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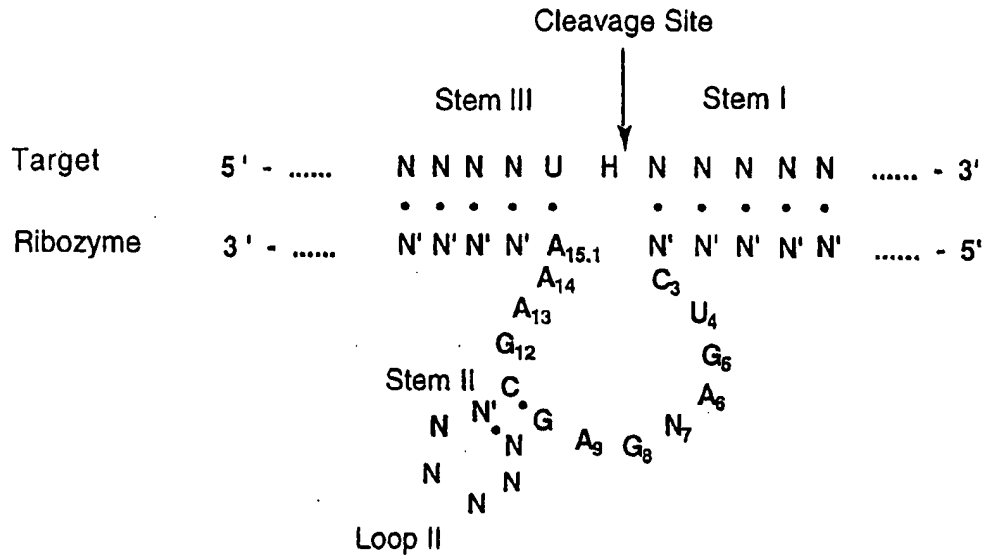

(57) Abstract

Enzymatic nucleic acid molecules which cleave EGFR RNA.

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DESCRIPTION

Enzymatic Nucleic Acid Treatment Of Diseases Or Conditions Related To Levels Of Epidermal Growth Factor Receptors

Background Of The Invention

The present invention concerns therapeutic compositions and methods for the treatment of cancer.

The present invention relates to therapeutic compositions and methods for the treatment or diagnosis of diseases or conditions related to EGFR expression levels, such as cancer. The following summary is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

The epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane glycoprotein consisting of an extracellular 'ligand' binding domain, a transmembrane region and an intracellular domain with tyrosine kinase activity (Kung et al., 1994). The binding of growth factors to the EGFR results in down regulation of the ligand-receptor complex, autophosphorylation of the receptor and other protein substrates, leading ultimately to DNA synthesis and cell division. The external ligand binding domain is stimulated by EGF and also by TGFα, amphiregulin and some viral growth factors (Modjtahedi & Dean, 1994).

The EGFR gene (c-erbB1), is located on chromosome 7, and is homologous to the avian erythroblastosis virus oncogene (v-erbB), which induces malignancies in chickens. The v-erbB gene codes for a truncated product that lacks the extracellular ligand binding domain. The tyrosine

kinase domain of the EGFR has been found to have 97% homology to the v-erbB transforming protein (Downward et al., 1984).

EGFR is overexpressed in a number of malignant human tissues when compared to their normal tissue counterparts (for review see Khazaie et al., 1993). The gene for the receptor is both amplified and overexpressed in a number of cancer cells. Overexpression of the EGFR is often accompanied by the co-expression of the growth factors EGF and TGFα, suggesting that an autocrine pathway for control of growth may play a major part in the progression of tumors (Sporn & Roberts, 1985).

Growth factors and their receptors may play a role in the development of human brain tumors. A high incidence of overexpression, amplification, deletion and structural rearrangement of the gene coding for the EGFR has been found in biopsies of brain tumors (Ostrowski et al., 1994). In fact the amplification of the EGFR gene in glioblastoma multiform tumors is one of the most consistent genetic alterations known, with the EGFR being overexpressed in approximately 40% of malignant gliomas (Black, 1991). It has also been demonstrated that in 50% of glioblastomas, amplification of the EGFR gene is accompanied by the co-expression of mRNA for at least one or both of the growth factors EGF and TNFα (Ekstrand et al., 1991).

The amplified genes are frequently rearranged and associated with polymorphism leading to abnormal protein products (Wong et al., 1994). The rearrangements that have been characterized usually show deletions of part of the extracellular domain, resulting in the production of an EGFR protein that is smaller in size. Three classes of deletion mutant EGF receptor genes have been identified in

glioblastoma tumors. Type I mutants lack the majority of the external domain, including the ligand binding site, type II mutants have a deletion in the domain adjacent to the membrane but can still bind ligands and type III, which is the most common and found in 17% of glioblastomas, have a deletion of 267 amino acids spanning domains I and II of the EGFR.

In addition to glioblastomas, abnormal EGFR expression has also been reported in a number of squamous epidermoid cancers and breast cancers (reviewed in Kung et al, 1994; Modjtahedi & Dean, 1994). Many patients with tumors that overexpress the EGFR have a poorer prognosis than those who do not (Khazaie et al., 1993). Consequently, therapeutic strategies which can potentially inhibit or reduce the aberrant expression of the EGFR receptor are of great interest as potential anti-cancer agents.

Summary Of The Invention

This invention relates to ribozymes, or enzymatic nucleic acid molecules, directed to cleave RNA species that are required for cellular growth responses. In particular, applicant describes the selection and function of ribozymes capable of cleaving RNA encoded by the receptor of epidermal growth factor (EGFR). Such ribozymes may be used to inhibit the hyper-proliferation of tumor cells in one or more cancers.

In the present invention, ribozymes that cleave EGFR RNA are described. Those of ordinary skill in the art will understand that from the examples described that other ribozymes that cleave target RNAs required for cell proliferation may be readily designed and are within the invention. Such RNAs may have at least 90% homology to EGFR in humans with a normal EGFR gene.

By "inhibit" is meant that the activity of EGFR or level of RNAs encoded by EGFR is reduced below that observed in the absence of the nucleic acid, particularly, inhibition with ribozymes preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention.

The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, minizyme, leadzyme, oligozyme or DNA enzyme, as used in the art. All of these terminologies describe nucleic acid molecules with enzymatic activity.

By "equivalent" RNA to EGFR is meant to include those naturally occurring RNA molecules associated with cancer in various animals, including human.

By "complementarity" is meant a nucleic acid that can form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

Seven basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, since the concentration of ribozyme necessary to affect a therapeutic treatment is lower. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a ribozyme.

Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other

separate RNA molecules in a nucleotide base sequence-specific manner. Such enzymatic RNA molecules can be targeted to virtually any RNA transcript, and efficient cleavage achieved in vitro (Zaug et al., 324, Nature 429 1986 ; Uhlenbeck, 1987 Nature 328, 596; Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Dreyfus, 1988, Einstein Quart. J. Bio. Med., 6, 92; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989).

Because of their sequence-specificity, trans-cleaving ribozymes show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 Ann. Rep. Med. Chem. 30, 285-294; Christoffersen and Marr, 1995 J. Med. Chem. 38, 2023-2037). Ribozymes can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

Ribozymes that cleave the specified sites in EGFR RNAs represent a novel therapeutic approach to treat diseases, such as cancer and other conditions. Applicant indicates that ribozymes are able to inhibit the activity of EGFR and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these sites in EGFR RNAs may be readily designed and are within the scope of this invention.

In one of the preferred embodiments of the inventions herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in

the motif of a hepatitis δ virus, group I intron, group II intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA. Examples of such hammerhead motifs are described by Dreyfus, supra, Rossi et al., 1992, AIDS Research and Human Retroviruses 8, 183; of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, Feldstein et al., 1989, Gene 82, 53, Haseloff and Gerlach, 1989, Gene, 82, 43, and Hampel et al., 1990 Nucleic Acids Res. 18, 299; of the hepatitis δ virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada et al., 1983 Cell 35, 849; Forster and Altman, 1990, Science 249, 783; Li and Altman, 1996, Nucleic Acids Res. 24, 835; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799; Guo and Collins, 1995, EMBO. J. 14, 363); Group II introns are described by Griffin et al., 1995, Chem. Biol. 2, 761; Michels and Pyle, 1995, Biochemistry 34, 2965; Pyle et al., International PCT Publication No. WO 96/22689; and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule (or multiple fragments of such molecules) of this invention is that it has a specific substrate binding site or arm(s) which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule (enzymatic portion).

By "enzymatic portion" is meant that part of the

ribozyme essential for cleavage of an RNA substrate.

By "substrate binding arm" is meant that portion of a ribozyme which is complementary to (i.e., able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme and target RNA together through complementary base-pairing interactions; e.g., ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding EGFR proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed

from DNA/RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs (e.g., antisense oligonucleotides, hammerhead or the hairpin ribozymes) are used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of the mRNA

structure. However, these nucleic acid molecules can also be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985 Science 229, 345; McGarry and Lindquist, 1986 Proc. Natl. Acad. Sci. USA 83, 399; SullengerScanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol, 66, 1432-41; Weerasinghe et al., 1991 J. Virol, 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res. 23, 2259). Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of EGFR activity in a cell or tissue.

By "related" is meant that the inhibition of EGFR RNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to

relevant tissues ex vivo, or in vivo through injection, infusion pump or sent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables III and IV. Examples of such ribozymes are also shown in Tables III and IV. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Thus, in a first aspect, the invention features ribozymes that inhibit gene expression and/or cell proliferation via cleavage of RNA expressed from the EGFR gene. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, cell proliferation is inhibited.

In a preferred embodiment, the enzymatic RNA molecules cleave EGFR mRNA and inhibit cell proliferation. Such ribozymes are useful for the prevention and/or treatment of cancer. Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to smooth muscle cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use

of a catheter, infusion pump or sent, with or without their incorporation in biopolymers. The ribozymes, similarly delivered, also are useful for inhibiting proliferation of certain cancers associated with elevated levels of the EGFR, particularly glioblastoma multiform. Using the methods described herein, other enzymatic RNA molecules that cleave EGFR and thereby inhibit tumor cell proliferation may be derived and used as described above. Specific examples are provided below in the Tables and figures.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit EGFR activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells explanted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review see Couture and Stinchcomb, 1996, TIG., 12, 510).

By "patient" is meant an organism which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which enzymatic nucleic acid molecules can be administered. Preferably,

a patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

These ribozymes, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with EGFR levels, the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art.

In a further embodiment, the described ribozymes can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described ribozymes could be used in combination with one or more known therapeutic agents to treat cancer.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in the tables III and IV (Seq ID NOs. 1-823 and 1759-1870. Examples of such ribozymes are also shown in Tables III and IV (Seq. ID Nos. 824-1758). Other sequences may be present which do not interfere with such cleavage.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2a is a diagrammatic representation of the

hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, Nature, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, Nature, 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, Nucl. Acids. Res., 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (i.e., n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, i.e., m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (i.e., r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (e.g., 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (i.e., o and p is each independently from 0 to any number, e.g., 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, i.e., without a connecting loop. The connecting loop when present may be a ribonucleotide with or without

modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 shows in vitro RNA cleavage activity of Amino ribozymes targeted against EGFR RNA. **a** Autoradiograph of the cleavage reaction. The reaction was performed in the presence of 50mM Tris.HCl (pH 7.5), 10mM $MgCl_2$ at 37°C as described below. Times of the reaction in minutes are given above the lanes. S0 represents intact substrate in Tris.HCl buffer without the addition of ribozyme at time 0. S1 represents intact substrate in Tris.HCl buffer at time 60min. +C represents a positive control of cleaved product only. Band S represents intact substrate, band P cleaved product and band D degradation; **b** Time course of cleavage. Bands from autoradiography were quantified by scanning densitometry and the fraction of substrate remaining plotted against time. inset. Semilog plots were used to determine the half life of the substrate ($t_{1/2}$ = 0.693 / k); **c** Autoradiograph showing reaction of the EGFR ribozyme against a non complementary substrate RNA. 40nM ribozyme was added to 1nM substrate in the presence of 50mM Tris.HCl (pH 7.5), 10mM $MgCl_2$ at 37°C. Band S refers to intact substrate and band P is cleaved product. Reaction times are given in minutes (unless stated otherwise). **C** represents intact substrate without the addition of ribozyme. +C represents cleaved product.

Figure 7 Representative examples of autoradiographs depicting the time course of cleavage reactions exhibited by EGFR ribozyme against it's target substrate under multiple turnover reactions. **a** In vitro activity of 10nM ribozyme with 300nM of 5' [32P] labeled substrate RNA; **b** In vitro activity of 10nM ribozyme with 1μM of 5'[32P] labeled substrate RNA. Reactions were performed in the presence of 50mM Tris.HCl (pH 7.5), 10mM $MgCl_2$ at 37°C as described below. Reaction times, in minutes, are given above the lanes. C represents intact substrate in Tris.HCl buffer without the addition of ribozyme. Band S refers to intact substrate and band P refers to cleaved product. **c** Kinetics of hammerhead cleavage reactions exhibited by the EGFR ribozyme. The initial rate of reaction (Vo,nM / min) is plotted versus substrate concentration. Ribozyme concentration was 10nM while substrate concentration varied as indicated. inset Eadie-Hofstee plot of this data.

Figure 8 shows a generic structure of chemically modified amino hammerhead ribozyme.

Figure 9 shows a generic structure of chemically modified C-allyl hammerhead ribozyme.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al., WO 93/23569; Sullivan et al., WO 93/23057; Thompson et al., WO 94/02595; Draper et al., WO 95/04818; McSwiggen et al., US Patent No. 5,525,468 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those

applications and synthesized to be tested in vitro and in vivo, as also described. Such ribozymes can also be optimized and delivered as described therein.

The sequence of human EGFR RNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables III and IV (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes were designed that could bind and were individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845; Scaringe et al., 1990 Nucleic Acids Res., 18, 5433; and Wincott et al., 1995 Nucleic Acids Res. 23, 2677-2684 and makes use of common nucleic acid protecting and coupling groups,

such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5 μmol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table **II** outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μL of 0.1 M = 16.3 μmol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μL of 0.25 M = 59.5 μmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer : detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I_2, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of EtOH:MeCN:H_2O/3:1:1, vortexed and the supernatant was then

added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA·HF/NMP solution (250 μL of a solution of 1.5mL N-methylpyrrolidinone, 750 μL TEA and 1.0 mL TEA·3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

Inactive hammerhead ribozymes were synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel, K. J., et al., 1992, Nucleic Acids Res., 20, 3252).

The average stepwise coupling yields were >98% (Wincott et al., 1995 Nucleic Acids Res. 23, 2677-2684).

Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51).

Ribozymes are modified to enhance stability and/or enhance catalytic activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992 TIBS 17, 34;

Usman et al., 1994 Nucleic Acids Symp. Ser. 31, 163; Burgin et al., 1996 Biochemistry 6, 14090). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott et al., supra) the totality of which is hereby incorporated herein by reference) and are resuspended in water.

The sequences of the ribozymes that are chemically synthesized, useful in this study, are shown in Tables III-IV. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables IV (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. Preferably, no more than 200 bases are inserted at these locations. The sequences listed in Tables III and IV may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes (which have enzymatic activity) are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., supra. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications

(base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases and/or enhance their enzymatic activity (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; and Burgin et al., supra; all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements are desired. (All these publications are hereby incorporated by reference herein.).

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of these properties in increased or not significantly (less that 10 fold) decreased in vivo compared to an all RNA ribozyme.

The enzymatic nucleic acid having chemical modifications which maintain or enhance enzymatic activity is provided. Such nucleic acid is also generally more resistant to nucleuses than unmodified nucleic acid. By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified ribozymes having a base substitution

selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in a cell and/or in vivo the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in a cell and/or in vivo even if activity over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Sullivan, et al., supra, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered ex vivo to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or sent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector.

Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U.S.A., 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisziewicz et al., 1993 Proc. Natl. Acad. Sci. U.S.A., 90, 8000-4;Thompson et al., 1995 Nucleic Acids Res. 23, 2259; Sullenger & Cech, 1993, Science, 262, 1566). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, supra).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves

mRNAs encoded by EGFR is inserted into a plasmid DNA vector or an adenovirus or adeno-associated virus DNA viral vector or a retroviral RNA vector. Viral vectors have been used to transfer genes and lead to either transient or long term gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, e.g., through incubation or inhalation in vivo or by direct application to cells or tissues ex vivo. Retroviral vectors have also been used to express ribozymes in mammalian cells (Ojwang et al., 1992 supra; Thompson et al., 1995 supra; Couture and Stinchcomb, 1996, supra).

In another preferred embodiment, the ribozyme is administered to the site of EGFR expression (e.g., tumor cells) in an appropriate liposomal vesicle.

Examples

Example 1: Identification of Potential Ribozyme Cleavage Sites in Human EGFR RNA

The sequence of human EGFR RNA was screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and potential hammerhead and/or hairpin ribozyme cleavage sites were identified. The sequences of these cleavage sites are shown in tables III and IV.

Example 2: Selection of Ribozyme Cleavage Sites in Human EGFR RNA

To test whether the sites predicted by the computer-

based RNA folding algorithm corresponded to accessible sites in EGFR RNA, 20 hammerhead sites were selected for analysis. Ribozyme target sites were chosen by analyzing genomic sequences of human EGFR (GenBank Accession No. X00588) and prioritizing the sites on the basis of folding. Hammerhead ribozymes were designed that could bind each target (see Figure 2C) and were individually analyzed by computer folding (Christoffersen et al., 1994 J. Mol. Struc. Theochem, 311, 273; Jaeger et al., 1989, Proc. Natl. Acad. Sci. USA, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Example 3: Chemical Synthesis and Purification of Ribozymes for Efficient Cleavage of EGFR RNA

Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the RNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described in Usman et al., (1987 J. Am. Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., supra, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G_5 and a U for A_{14}

(numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes were synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51). All ribozymes were modified to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Wincott et al., supra; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in Table III and IV.

Example 4: Ribozyme Cleavage of EGFR RNA Target

Twenty hammerhead-type ribozymes targeted to the human EGFR RNA were designed and synthesized to test the cleavage activity in vitro. The target sequences and the nucleotide location within the EGFR mRNA are given in Table III. All hammerhead ribozymes were synthesized with binding arm (Stems I and III; see Figure 2C) lengths of seven nucleotides. The relative abilities of a HH ribozyme to cleave human EGFR RNA is summarized in Figure 6 and 7.

Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay was prepared by in vitro transcription in the presence of [a-^{32}P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further

purification. Alternately, substrates were 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays were performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM $MgCl_2$) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays were carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, i.e., ribozyme excess. The reaction was quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample was heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage were visualized on an autoradiograph of the gel. The percentage of cleavage was determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

Single Turnover Reaction: Alternately, Cleavage reactions were carried out in 50mM Tris.HCl, pH 7.5 and 10mM $MgCl_2$ at 37°C. In order to disrupt aggregates that can form during storage, unlabeled ribozyme and 5'end labeled substrate were denatured and renatured separately in standard cleavage buffer (50mM Tris.HCl, pH 7.5) by heating to 90°C for 2 minutes and allowed to equilibrate to the reaction temperature of 37°C for 15 minutes. Each RNA solution was then adjusted to a final concentration of 10mM $MgCl_2$ and incubated at 37°C for a further 15 minutes. Cleavage reactions were initiated by combining the ribozyme and the substrate samples to the required

concentrations in a final volume of 100μl. Ribozyme concentration was 40nM and substrate concentration was 1nM. The reaction was also repeated using double (2nM) and half (0.5nM) the concentration of substrate to verify that the reaction was indeed performed under single turnover conditions. Aliquots of 10μl were removed at appropriate time intervals between 0 and 120 minutes and quenched by adding an equal volume of formamide loading buffer (9:1 (v:v) formamide:1x TBE) and frozen on dry ice. Product and substrate were separated by denaturing 20% polyacrylamide (7M urea) gel electrophoresis. To determine the fraction of cleavage, substrate and product bands were located by autoradiography of wet gels and quantified by densitometry of these autoradiograms. Autorads were scanned using an AGFA focus scanner connected to a Macintosh computer and images were saved as TIFF files. The programme NIH Image 1.58 (Division of Computing and Research Technology, NIH, Bethesda, USA) was used to plot and quantify the band intensities. In addition, the relevant bands were excised from the gel and quantified by scintillation counting of the slices cut from the gel (Packard Tricarb 2000 CA liquid scintillation analyser).

Reaction rate constants (k) were obtained from the slope of semilogarithmic plots of the amount of substrate remaining versus time. The activity half time t1/2 was calculated as 0.693/ k. Each rate constant was determined from duplicate experiments.

In order to show the specificity of cleavage demonstrated under the above conditions, the experiment was repeated using a different substrate, relating to another site along the human EGFR mRNA. All conditions remained as described above except

samples were taken over a longer time period i.e.at intervals spanning over 24 hours rather than over 2 hours.

Multiple Turnover Reactions: The kinetic characteristics of ribozyme RPI.4782 were determined from Eadie - Hofstee plots obtained from initial velocities with multiple turnovers done with 5' 32P labeled substrate. Cleavage reactions were carried out in 50mM Tris.HCl, pH7.5 and 10mM $MgCl_2$ at 37°C. Stock solutions of 100nM ribozyme and 500nM - 2uM substrate RNA were prepared in 50mM Tris.HCl, pH 7.5, preheated separately at 0°C for 2 minutes and cooled to 37°C for 15 minutes. After $MgCl_2$ was added to each of these solutions to a final volume of 10mM, a further incubation period of 15 minutes at 37°C took place. Cleavage reactions were performed in a final volume of 100μl with a concentration of 10nM ribozyme and concentrations of substrate between 100nM and 1μM. Reactions were initiated by the addition of ribozyme stock solution to substrate. Aliquots of 10μl were taken at time intervals between 0 and 120 minutes, quenched by adding an equal volume of formamide loading buffer and frozen on dry ice. Intact substrate and products of cleavage were separated by electrophoresis on a 20% polyacrylamide / 7M urea denaturing gel and were detected by autoradiography. The degree of cleavage at each time point was quantified by scanning densitometry of the resulting autoradiogram. Initial rates of reaction were measured at eight substrate concentrations and values of Kcat and Km were determined using Eadie-Hofstee plots.

As shown in Figure 6 and 7, Amino hammerhead ribozymes (RPI.4782) targeted against EGFR RNA cleaved their target RNAs in a sequence-specific manner the cleavage rates appeared to follow saturation kinetics with respect to concentration of substrate. Cleavage rates were first order at low substrate concentrations, however, as the concentration of substrate increased, the reaction

rates leveled off suggesting that ribozymes were effectively saturated with substrate. These results indicate that the cleavage reactions were truly catalytic and were therefore amenable to analysis using Michaelis Menten rate equation. From a Eadie-Hofstee plot the kinetic parameters Km and Kcat were determined; ribozyme exhibited a Km value of 87nM and a Kcat value of 1.2 min^{-1}.

Under single turnover conditions, ribozyme RPI.4782 exhibited rapid cleavage of it's target sequence, the half life of the substrate being only 7 minutes. The high activity of this ribozyme is in agreement with the findings of Beigelman et al. (1995c). They reported that a ribozyme modified in the same manner as RPI.4782 exhibited almost wild type activity, with the half life of the substrate being only 3 minutes. Although cleavage was slightly slower than that demonstrated by Beigelman et al. (1995c), these findings clearly demonstrate that ribozyme RPI.4782 is able to cleave it's target in a highly efficient manner.

When the experiment was repeated using a different, non complementary, substrate sequence, no cleavage products were evident (figure 3.3), demonstrating the sequence specificity of this molecule.

To assess more precisely the activity of ribozyme Amino ribozyme (RPI.4782), the kinetic parameters K_M and k_{cat} were determined under multiple turnover conditions. The results indicate that the cleavage reaction was truly catalytic with a turnover rate (K_{cat}) of 1.2 min^{-1} and a K_M value of 87nM (figure 6 and 7). These results fall in line with typical values reported for the hammerhead ribozyme of 1-2 min^{-1} and 20-200nM for Kcat and Km respectively (Kumar et al., 1996). Direct comparisons are difficult, however, since many factors including base sequence, length of substrate binding arms and varying chemical

modifications can have an effect on these kinetic parameters (Fedor & Uhlenbeck, 1992).

Example 5: Stability of EGFR Ribozymes in Fetal Calf Serum.

To assess the stability of the chemically modified ribozyme, a comparative stability study was carried out in 100% foetal calf serum (Gibco, Paisley,U.K.) at 37°C. Degradation profiles of 5' and internally [^{32}P] labeled ribozyme were compared to those of 5'-end [^{32}P] labeled phosphoodiester (PO), phosphorothioate (PS) oligodeoxynucleotides and unmodified RNA.

Synthesis / labeling: 37mer PO and PS oligonucleotides were synthesized on an automated DNA synthesizer (model 392, Applied Biosystems, Warrington, U.K.) using standard phosphoramide chemistry (section 2.2.1). The chemically modified 37mer ribozyme (Amino Hammerhead Ribozyme; Figure 8) and the 15mer unmodified all RNA substrate were synthesized as described above. Ribozymes and oligonucleotides were radiolabelled with [^{32}P] ATP and purified on 20% polyacrylamide gel as previously described.

Degradation study conditions: Radiolabelled ribozymes/ oligonucleotides were incubated in 100 μl of FCS at 37°C to give a final concentration of 200nM. 10ul aliquots were removed at timed intervals, mixed with a loading buffer containing 80% formamide, 10mM EDTA (pH8.0), 0.25% xylene cyanol, 0.25% bromophenol blue, and frozen at -20°C prior to gel loading. Degradation profiles were analyzed by 20% polyacrylamide (7M urea) gel electrophoresis and autoradiography.

A comparative stability study was undertaken in 100% fetal calf serum (FCS) to compare the degradation profiles

of 5' end labeled and internally labeled amino ribozyme to those of 5'end labeled unmodified RNA substrate, phosphodiester (PO) and phosphorothioate (PS) oligodeoxynucleotides. The chemical modifications of the amino ribozyme resulted in a substantial increase in nuclease resistance over that of the unmodified substrate. The half life ($t_{50\%}$) of the internally labeled ribozyme was approximately 20 hours whereas the substrate was completely degraded within the time that it took to add the RNA to serum, mix and quench the reaction ($t_{50\%}$ < 1min). It was interesting to note that although the patterns of degradation were clearly different for the internally labeled ribozyme (figure 3.6a) and the 5' end labeled ribozyme, the kinetics of degradation were strikingly similar. ($t_{50\%}$ of ≈ 20 hours for both).

A comparison of ribozyme degradation and oligodeoxynucleotide degradation was also performed. The chemically modified ribozyme appeared to be more stable in FCS than either the PO oligonucleotide or the PS oligonucleotide; the approximate half lives being 10 minutes and 5 hours respectively. It must be noted, however, that the apparent degradation products migrated to the position of free phosphate. This suggests that dephosphorylation (removal of [^{32}P] label) occurred, resulting in a progressive increase in free phosphate concentration with time.

There is no doubt, however, that the findings of this study show that the chemical modifications applied to ribozyme result in an extremely stable structure. Under the conditions of this experiment amino ribozyme proved to be the most stable to nuclease mediated degradation in fetal calf serum.

Example 6: Ribozymes uptake studies

Cell Culture Techniques U87-MG cell line was purchased from the European Cell Culture Collection, Porton Down, U.K. These human glioblastoma astrocytoma cells were originally derived from a grade 3 malignant glioma by explant technique (Poten et al.,1968). A431 cells were derived from a vulval carcinoma and expresses the EGFR at levels 10 to 50 fold higher than seen in other cell lines (Ullrich et al., 1984).

The cell lines U87-MG and Raw 264.7 were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented by 10% v/v foetal bovine serum (FBS), 1% penicillin/streptomycin and 1% v/v L-glutamine (all supplied from Gibco, Paisley, U.K.). The same media, without the addition of the foetal bovine serum, was used in the stability and uptake studies. A431 cells were maintained under the same conditions except glutamine was added to a final concentration of 2% v/v. CaCo-2 cells were kindly cultured and plated by Vanessa Moore in DMEM, 10% FBS, 1% non essential amino acids, 1% penicillin/ streptomycin, and 1% L-glutamine.

Cells were cultured in $75cm^3$ plastic tissue culture flasks (Falcon, U.K.) with 25ml of the respective media. The cultures were incubated at 37°C in a humidified (95%) atmosphere of 5% CO2 in air. Stock cultures were maintained by changing the media every 48 hours and passaged (1:5) when confluent (after approximately 4 days). Passaging was carried out using the following procedure:

The media was removed and the cells washed with 10ml of phosphate-buffered saline solution (PBS). Following this, 5ml of 2x Trypsin/EDTA (0.25% w/v trypsin, 0.2% disodium ethylenediamine tetraacetate in PBS, pH 7.2) was added and the flasks incubated at 37°C for 5minutes. The

flasks were tapped to dislodge the cell monolayer from the bottom and fresh media was added to neutralize the trypsin. The cells were split as required and media added to a final volume of 25ml.

For long term storage, frozen stock cultures were prepared in the following manner:

Stock cultures were trypsinised as described and neutralized with the addition of 10ml of DMEM media. The cell suspension was then transferred to a 15ml universal tube (Falcon, U.K.) and centrifuged for 3 minutes at 350 revolutions per minutes. The supernatant was decanted and the cell pellet was resuspended in 1ml of freezing media (10% DMSO, 90% heat inactivated foetal calf serum) and transferred to a 2ml screw capped cryovial (Costar, U.K.). The ampule was then placed in the freezing head of a liquid nitrogen freezer for 4-6 hours before being transferred into liquid nitrogen (-196°C) cell bank. When required, the cells were recovered by rapid thawing at 37°C and gradual dilution with DMEM media before seeding in $25cm^3$ flasks (Falcon, U.K.).

The viable cell density of stock cultures was measured by haemocytometry using a trypan blue exclusion test. 100μl of trypan blue (4mg ml^{-1}) was mixed with 400μl of cell suspension (1:1.25 dilution). A small amount of the trypan blue-cell suspension was transferred to the counting chamber of a Neubauer haemocytometer, with depth of 0.1mm and area $1/400mm^2$ (Weber Scientific International Ltd, U.K.). The cells were counted in the 5 large squares of the haemocytometer using a light microscope. Since live cells do not take up the trypan blue dye, while dead cells do, the number of viable (unstained) cells were counted. The cell density was calculated using the following equation:

cells ml^{-1} = average count per square x 10^4 x 1.25 (dilution factor of trypan blue)

Cell Association Studies: A series of experiments were conducted to examine the mechanism of uptake of the ribozyme in the U87-MG glioblastoma cell line. The following general experimental procedure was used throughout these studies unless otherwise stated.

Synthesis/ labelling: Prior to use in uptake studies, the 37mer ribozyme was internally labeled with 32P as previously described (section 2.3.2) and purified by 20% native polyacrylamaide gel electrophoresis. [14C]

Mannitol (specific activity 56mCi / mmol) was purchased from Amersham (Amersham, U.K.).

Uptake study procedure: U87-MG cells were cultured on plastic 24-well plates (Falcon, U.K.). Confluent stock cultures were trypsinised and the cell density of the stock suspension diluted to 0.5 x 10^5 cells ml^{-1} with DMEM media. Each well was seeded with 2ml of the diluted cell suspension to give a final concentration of $1x10^5$ well-1. The plates were incubated at 37°C in a humidified (95%) atmosphere of 5% CO_2 in air. After approximately 20 - 24 hours, the cell monolayers had reached confluency and were then ready for uptake experiments. The media was then removed and the monolayer carefully washed twice with PBS (2 x 1ml x 5min) to remove any traces of serum. The washing solution was aspirated and replaced with 200μl of serum free DMEM media containing the radiolabelled ribozyme. Both PBS and serum free media were equilibrated at 37°C for 1hour prior to use. The plates were incubated at 37°C, unless otherwise stated, in a dry environment for the duration of the experiment. Once incubated for the desired period of time, the apical media was carefully collected and their radioactive content assessed by liquid

scintillation counting (LSC) The cells were then washed 3 times * (3 x 0.5ml x 5min) with ice cold PBS/ sodium azide (0.05% w/v NaN_3 / PBS) to inhibit any further cellular metabolism and remove any ribozyme loosely associated with the cell surface. The washings were collected and their radioactive content determined by LSC. Cell monolayers were solubilized by shaking with 0.5ml of 3% v/v Triton X100 (Aldrich Chemical Company, Gillingham, UK) in distilled water for 1 hour at room temperature. The wells were washed twice more (2 x 0.5ml) with Triton X-100 to ensure that all the cells had been harvested and the radioactivity content of the cellular fraction determined by LSC. Unless otherwise indicated, all experiments were performed at a final concentration of 0.01μM 32P internally labeled riboxyme and incubated for a period of 60 minutes.

The uptake of Amino ribozymes were compared in different cell lines. The results show that cellular association of these ribozymes ranged from 0.325 ± 0.021 ng/10^5 cells in intestinal epithelial cells to 1.09 ± 0.207 ng/10^5 cells in the macrophage cell line.

The ability of ribozymes to penetrate the cell membrane and the mechanism of entrance are important considerations in developing ribozymes as therapeutics. The mechanisms by which oligodeoxynucleotides enter cells has been well documented (for review see Akhtar & Juliano, 1991) and include the involvement of fluid phase, adsorptive and receptor mediated endocytosis. The mechanism and extent of uptake is dependent on many factors including oligonucleotide type and length and cell line studied. In contrast, however, no mechanism of cellular uptake has yet been described for ribozymes and ribonucleotides. In order to investigate the means of

uptake of ribozyme RPI.4782 in glioma cells, a series of cellular association studies were performed in the human glioma derived cell line, U87-MG.

The cellular association of ribozyme RPI.4782 to U87-MG cells appeared to be biphasic, with a rapid initial phase continuing for approximately two hours followed by a slower second phase. The cellular association of oligonucleotides has been shown to be a dynamic process representing both uptake and efflux processes (Jaroszewski & Cohen, 1990). Consequently, the plateauing seen in the second phase could represent an equilibrium of both uptake and exocytosis of ribozyme. The uptake of ribozyme RPI.4782 was strongly dependent on temperature, suggesting that an active process is involved. In addition, the metabolic inhibitors, sodium azide and 2-deoxyglucose significantly inhibited cellular association by 66%, demonstrating that ribozyme uptake was also energy dependent.

The energy and temperature dependency of cellular association of this ribozyme in U87-MG cells are characteristic of an active process, indicating that the mechanism of uptake is via endocytosis. These findings do not, however, distinguish whether fluid phase endocytosis or receptor mediated endocytosis is involved; since both mechanisms will be effected by these parameters (Beltinger et al., 1994). In order to evaluate the pathway of internalization, the uptake of a fluid phase marker, [14C] mannitol, was measured to determine the extent of pinocytosis in U87-MG cells. The basal rate of pinocytosis in these cells remained extremely low throughout the time period tested and it is unlikely, therefore, to account for a significant fraction of ribozyme uptake in this cell line.

To investigate whether ribozyme RPI.4782 is taken up

into U87-MG cells by receptor mediated endocytosis a self competition study was conducted. Ribozyme uptake was found to be significantly inhibited by competition with unlabeled ribozyme. This demonstrates that cellular association was concentration dependent and suggests that the dominant uptake mechanism is via receptor mediated endocytosis.

Receptor mediated endocytosis involves the internalization of molecules via specific membrane protein, cell surface receptors. Consequently, a proteolytic enzyme such as trypsin or pronase® can be used to determine the extent to which membrane proteins mediate uptake (Beck et al., 1996; Shoji et al., 1991; Wu-pong et al., 1994). In a study investigating the cellular association of oligonucleotides in intestinal CaCo-2 cells, Beck et al. (1996) reported a 50% reduction of uptake upon cell surface washing with pronase, while 60% of oligonucleotide uptake was reported to be trypsin sensitive in Rauscher Red 5-1.5 ertythroleukemai cells (Wu-Pong et al., 1994). To further characterize ribozyme uptake, the effects of the endocytosis inhibitor, phenylarsine oxide and the endosomal alkalinizers, chloroquine and monensin could be studied (Loke et al., 1989; Wu-Pong et al., 1994).

To determine whether specific binding sites are involved in the uptake of ribozyme RPI.4782 in U87-MG cells, competition studies are required to evaluate the effect on ribozyme uptake by competitors such as oligonucleotides, ATP and other polyanions, such as dextran sulphate and heparin. The cellular association of ribozyme RPI.4782 to U87-MG cells was also found to be pH dependent. In fact a decrease in pH from pH 8 to pH 5 resulted in a significant increase in cellular association. The effect of pH on ribozyme partition

coefficients had not as yet been undertaken in order to determine whether the increase in cellular association was due to an increase in the partition coefficient of the ribozyme, at low pH conditions. The increase of cellular association at low pH is in agreement with the work of Goodarzi et al. (1991) and Kitajima et al. (1992) who found that cellular association of oligonucleotides also increased under acidic conditions. It has been postulated that enhanced binding could be due to the presence of a 34kDa membrane protein receptor that functions around pH 4.5 (Goodarzi et al., 1991). In addition, the α amino group of lysine, the guanidium group of arginine and protonated imidazole of histidine have been suggested to be possible oligonucleotide binding sites (Blackburn et al., 1990). Histidine, having a pKa of 6.5 is susceptible to protonation over a pH range of 7.2 to 5.0. Therefore, the enhanced affinity of ribozyme RPI.4782 to U87-MG cells at pH 5.0 could be due to protonation of histidine residues present at the binding site.

In general these observations suggest that the pathway of cellular uptake of ribozyme involves an active cellular process; indications are that the predominant mechanism of uptake is via receptor mediated endocytosis.

Example 7: Ribozyme stability in U87-MG Cells

In order to ensure that the results obtained from the uptake studies represented cell association of **intact** 37mer ribozyme and not degraded ribozyme or free [^{32}P] label, the stability of this ribozyme,when incubated with U87 cells ,was examined.

U87-MG cells were seeded onto 24 well-plates as previously described and used approximately 24 hours post seeding. Internally [^{32}P] labeled ribozyme RPI.4782 was added to 200μl of serum free media to give a final

concentration of 10nM. 10μl aliquots of the apical solution were collected at variable time points over a period of 4 hours, mixed with an equal volume of formamide loading buffer (9:1 v/v formamide: 1x TBE) and stored at -20C. Prior to gel loading, the samples were heated to 100°C for 5 minutes and separated on 7M urea / 20% acrylamide gels; bands were detected by autoradiography of wet gels.

For comparative purposes, the stability profiles of 5' labeled ribozyme RPI.4782, 5' end labeled all RNA 15mer substrate, and 5' end labeled 37mer PO and PS oligodeoxynucleotides were also measured under the same conditions.

To ensure that any findings obtained from uptake studies represented the cellular association of intact 37mer ribozyme and not that of shorter degraded fragments or free [^{32}P] label, the degradation of 5'-end and internally [^{32}P] labeled ribozyme was examined when exposed to U87-MG cells. For comparative purposes, the stability profile of an unmodified RNA substrate was also measured under the same conditions. The chemically modified ribozyme remained largely intact throughout a four hour incubation period. While no degradation was evident from the internally labeled sample, the 5'-end labeled ribozyme did exhibit some degradation after 120 minutes. This indicates that 5' dephosphorylation occurred in the latter case. In contrast, however, the unmodified RNA substrate was completely degraded within 10 minutes incubation with the U87-MG cell monolayer. The ribozyme was clearly protected from cellular nucleuses by the chemical modifications previously described.

Optimizing Ribozyme Activity

Sullivan, et al., supra, describes the general

methods for delivery of enzymatic RNA molecules. The data presented in Examples above indicate that different cationic lipids can deliver active ribozymes to smooth muscle cells. Experiments similar to those performed in above-mentioned Examples are used to determine which lipids give optimal delivery of ribozymes to specific cells. Other such delivery methods are known in the art and can be utilized in this invention.

The proliferation of smooth muscle cells can also be inhibited by the direct addition of chemically stabilized ribozymes. Presumably, uptake is mediated by passive diffusion of the anionic nucleic acid across the cell membrane. In this case, efficacy could be greatly enhanced by directly coupling a ligand to the ribozyme. The ribozymes are then delivered to the cells by receptor-mediated uptake. Using such conjugated abducts, cellular uptake can be increased by several orders of magnitude without having to alter the phosphodiester linkages necessary for ribozyme cleavage activity.

Alternatively, ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres.

The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or sent. Alternative routes of delivery include, but are not limited to, intramuscular injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., supra which have been incorporated by

reference herein.

Chemical modifications, ribozyme sequences and ribozyme motifs described in this invention are meant to be non-limiting examples, and those skilled in the art will recognize that other modifications (base, sugar and phosphate modifications) to enhance nuclease stability of a ribozyme can be readily generated using standard techniques and are hence within the scope of this invention.

Use of Ribozymes Targeting EGFR

Overexpression of the EGFR has been reported in a number of cancers (see above). Thus, inhibition of EGFR expression (for example using ribozymes) can reduce cell proliferation of a number of cancers, in vitro and in vivo and can reduce their proliferative potential.

Ribozymes, with their catalytic activity and increased site specificity (see above), are likely to represent a potent and safe therapeutic molecule for the treatment of cancer. In the present invention, ribozymes are shown to inhibit smooth muscle cell proliferation and stromelysin gene expression. From those practiced in the art, it is clear from the examples described, that the same ribozymes may be delivered in a similar fashion to cancer cells to block their proliferation. These ribozymes can be used in conjunction with existing cancer therapies.

Gliomas are the most common primary tumors arising from the brain, in fact each year malignant gliomas account for approximately 2.5% of the deaths from cancer (Bruner, 1994). These gliomas are morphologically and biologically heterogeneous and include neoplasms derived from several cell types. Astrocytomas form the largest single group among the primary tumors (75-90%) which also

includes oligodendrogliomas, ependymomas and mixed gliomas (Bruner, 1994). Distinct histological features allow astrocytomas to be graded into levels of anaplasia, the most widely used today involves a three tiered grading system (Ringertz, 1950) dividing astrocytomas into low grade astrocytomas, anaplastic astrocytomas and glioblastomas.

The most malignant and frequently occurring form, glioblastoma multiform (GBM), accounts for approximately one third of all primary brain tumors (Wong et al., 1994). This tumor is so undifferentiated that it cell of origin remains obscure, however most examples are generally thought to arise from astrocytes because glial fibrillary acidic protein (GFAP), a histological marker for astrocytes, can be identified in the cell cytoplasm.

The histological morphology of glioblastoma can be highly variable, confirming the name "multiforme".

The characteristic features of glioblastoma multiform is tumor necrosis. The individual cells may be small with a high nuclear / cytoplasmic ratio or very large and bizarre with abundant eosinophilic cytoplasm. The small cells are the more proliferative ones and show a more aggressive course. In fact some glioblastomas are so highly cellular that the population of small anaplastic cells stimulates primitive neuroectodermal tumors such as medulloblastoma. These small cells often appear to condense around areas of tumor necrosis forming characteristic 'pseudopalisades". They also have

the propensity to infiltrate the brain extensively, giving the appearance of multifocal gliomas.

Despite advances in many areas of cancer research and treatment, glioblastoma multiform almost always proves fatal, with a median survival rate of less than one year and a 5 year survival rate of 5.5% or less (Martuza et

al., 1991). At present, no therapeutic modality has substantially changed the outcome of patients with glioblastoma. Characteristics of this type of tumor, including it's invasive nature, it's ability to spread locally and distantly while avoiding recognition by the immune system, it's relative resistance to radiation and a high local recurrence rate, limit the success of conventional therapy. The effective treatment of glioblastoma multiform, therefore, presents a tremendous challenge.

The current methods of treatment used in the management of malignant gliomas are briefly reviewed.

Surgery: The cornerstone of therapy for glioblastoma multiform tumors has been surgery. The use of microsurgical techniques, intraoperative ultrasonic aspiration, electrophysiologic monitoring and lasers make the surgical procedure safe and accurate (Kornblith et al., 1993). Although surgery does improve the survival of patients with glioblastoma multiform, the inability to surgically remove eloquent areas of cerebral cortex invaded by the tumor render such ablative technologies of only modest value.

Radiotherapy: Malignant gliomas such as glioblastoma multiform exhibit an extraordinary resistance to radiotherapy and as a consequence the effectiveness of this form of treatment is limited. The sensitivity of the surrounding, unaffected, brain limits the dose that can safely be delivered to 60Gy (Leibel et al., 1994), which is well below the level required to completely eradicate the primary tumor in the majority of patients. In addition, whole brain radiotherapy does not prevent local tumor recurrence. The effective use of more localized forms of radiotherapy, such as radiosensitizers and radiosurgical techniques, are at present under review.

Chemotherapy: Chemotherapy has been shown to be effective adjuncts to surgery and radiotherapy in the treatment of cancer. Unfortunately, however, chemotherapy has had a limited impact on survival in patients with high grade astrocytomas. A report published in 1993 determined that adding chemotherapy to surgery and radiation improved the median survival duration in these patients from 9.4 to 12 months (Fine et al., 1993).

Generally, the relatively lipid soluble and non ionized nitrosourea drugs; e.g. carmustine, lomustine, semustine and nimustine, have proved to be the most active single chemotherapy agents for treating malignant astrocytomas (Lesser & Grossman, 1994). New drugs continue to enter clinical trials in patients with glioblastoma; none so far, however, have substantially prolonged a patient's life span. A myriad of physiological and biological factors such as the blood brain barrier, heterogeneous and resistant tumor cell populations and unacceptable toxicities have limited the efficacy of these agents.

Different routes of administration have been used to overcome the impenetrability of the blood brain barrier. A unique delivery system has been reported (Brem et al., 1991) which incorporates biodegradable polymers impregnated with chemotherapy agents. These polymers are placed topically at the resection site and slowly release the drugs as they degrade. Direct injection into tumors may also be useful as a means to deliver the highest dose to the tumor site without systemic exposure.

Immunotherapy: Glioblastoma multiform is an appropriate target for immunological directed therapy. Studies have revealed that sera from patients with GBM stimulates little or no humoral response. A realistic approach, therefore, is to stimulate a stronger immune

response in glioblastoma patients. Although this approach looks promising in theory, as yet no effective means of stimulating a clinically immune response has been identified. The most promising avenue, through the use of lymphokine activated killer (LAK) cells and interleukin - 2, has been limited by lack of tumor specific cell homing and difficulties with LAK cell delivery and toxicity.

Advances in the understanding of the molecular basis of cancer has now made it possible to design molecules that specifically interact with cancer cells. The most promising modes of therapy for the treatment of GBM, therefore, may lie with molecular based technologies which employ genetic interventions to alter the properties or behaviour of specific cells.

In fact ,glioblastoma multiform tumors are ideal candidates for this type of therapy since they rarely metastasize, are accessible to direct delivery techniques and can be precisely monitored by MRI and CT scans. The tumor cells may also divide rapidly, which enables agents such as retroviruses to infect the cells and synthesize genes leading to tumor cell destruction. (Kornblith et al., 1993).

Many detailed cytogenetic studies have been performed on malignant gliomas and these reveal commonly occurring abnormalities (Bigner & Vogelstein, 1990). For example, approximately 80% of malignant gliomas have gains of one or more copies of chromosome 7 and approximately 60% show a loss of chromosome 10. In addition, one of the most consistent genetic abnormalities is the presence of double minute chromosomes (DMs). Double minute chromosomes refer to small portions of chromosomes which are paired but lack a centromere; they are the karyotypic manifestation of gene amplification. The presence of such DMs have been found in over 50% of glioblastomas, with some tumors

possessing 50 - 100 copies of DMs per cell (Ostrowski et al., 1994). This indicates that gene amplification in a cancer cell is a key method of increasing a certain amount of protein.

Studies have revealed that a number of genes are amplified in glioblastoma tumors including the genes for: the epidermal growth factor receptor (EGFR); .c-myc, ros-1, myb, and gli (Ostrowski et al., 1994; Wong et al., 1994). Consequently many target areas exist for the future development of novel forms of therapy in the treatment of glioblastoma multiform.

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Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of EGFR RNA in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the

possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with EGFR related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., EGFR) is adequate to establish risk. If

probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

Table I

Table 1:

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-OH of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintainance of the active structure [1].
- Over 300 known members of this class. Found as an intervening sequence in Tetrahymena thermophila rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [2,3].
- Complete kinetic framework established for one ribozyme [4,5,6,7].
- Studies of ribozyme folding and substrate docking underway [8,9,10].
- Chemical modification investigation of important residues well established [11,12].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however,

Table I

the Tetrahymena group I intron has been used to repair a "defective"

- β-galactosidase message by the ligation of new β-galactosidase sequences onto the defective message [13].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [14].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3'-OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [15,16]
- Important phosphate and 2' OH contacts recently identified [17,18]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [19,20].
- Sequence requirements not fully determined.
- Reaction mechanism: 2'-OH of an internal adenosine generates cleavage products with 3'-OH and a "lariat" RNA containing a 3'-5' and a 2'-5' branch point.

Table I

- Only natural ribozyme with demonstrated participation in DNA cleavage [21,22] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [23].
- Important 2' OH contacts beginning to be identified [24]
- Kinetic framework under development [25]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [26].
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme
(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5'of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.

Table I

- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures []
- Minimal ligation activity demonstrated (for engineering through in vitro selection) []
- Complete kinetic framework established for two or more ribozymes [].
- Chemical modification investigation of important residues well established [].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [27,28,29,30]
- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through in vitro selection [31]
- Complete kinetic framework established for one ribozyme [32].

Table I

- Chemical modification investigation of important residues begun [33,34].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [35].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [36].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [37]

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Table II

Table II: 2.5 µmol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 µL	2.5
S-Ethyl Tetrazole	23.8	238 µL	2.5
Acetic Anhydride	100	233 µL	5 sec
N-Methyl Imidazole	186	233 µL	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table III

TABLE III: Human EGF-R Hammerhead Ribozyme and Target Sequences

nt. Position	Substrate	Seq. ID NOs.	Ribosyme	Seq. ID NOs.
19	GCCGGAGUC CCGAGCUA	1	UAGCUCGG CUGAUGA X GAA ACUCCGGC	824
27	CCCGAGCUA GCCCCGGC	2	GCCGGGGC CUGAUGA X GAA AGCUCGGG	825
70	GGCCACCUC GUCGGCGU	3	ACGCCGAC CUGAUGA X GAA AGGUGGCC	826
73	CACCUCGUC GGCGUCCG	4	CGGACGCC CUGAUGA X GAA ACGAGGUG	827
79	GUCGGCGUC CGCCCGAG	5	CUCGGGCG CUGAUGA X GAA ACGCCGAC	828
89	GCCCGAGUC CCCGCCUC	6	GAGGCGGG CUGAUGA X GAA ACUCGGGC	829
97	CCCCGCCUC GCCGCCAA	7	UUGGCGGC CUGAUGA X GAA AGGCGGGG	830
137	CCCUGACUC CGUCCAGU	8	ACUGGACG CUGAUGA X GAA AGUCAGGG	831
141	GACUCCGUC CAGUAUUG	9	CAAUACUG CUGAUGA X GAA ACGGAGUC	832
146	CGUCCAGUA UUGAUCGG	10	CCGAUCAA CUGAUGA X GAA ACUGGACG	833
148	UCCAGUAUU GAUCGGGA	11	UCCCGAUC CUGAUGA X GAA AUACUGGA	834
152	GUAUUGAUC GGGAGAGC	12	GCUCUCCC CUGAUGA X GAA AUCAAUAC	835
172	AGCGAGCUC UUCGGGGA	13	UCCCCGAA CUGAUGA X GAA AGCUCGCU	836
174	CGAGCUCUU CGGGGAGC	14	GCUCCCCG CUGAUGA X GAA AGAGCUCG	837
175	GAGCUCUUC GGGGAGCA	15	UGCUCCCC CUGAUGA X GAA AAGAGCUC	838
197	GCGACCCUC CGGGACGG	16	CCGUCCCG CUGAUGA X GAA AGGGUCGC	839
219	GCAGCGCUC CUGGCGCU	17	AGCGCCAG CUGAUGA X GAA AGCGCUGC	340
240	GCUGCGCUC UGCCCGGC	18	GCCGGGCA CUGAUGA X GAA AGCGCAGC	841
253	CGGCGAGUC GGGCUCUG	19	CAGAGCCC CUGAUGA X GAA ACUCGCCG	842
259	GUCGGGCUC UGGAGGAA	20	UUCCUCCA CUGAUGA X GAA AGCCCGAC	843
276	AAGAAAGUU UGCCAAGG	21	CCUUGGCA CUGAUGA X GAA ACUUUCUU	844
277	AGAAAGUUU GCCAAGGC	22	GCCUUGGC CUGAUGA X GAA AACUUUCU	845
292	GCACGAGUA ACAAGCUC	23	GAGCUUGU CUGAUGA X GAA ACUCGUGC	846
300	AACAAGCUC ACGCAGUU	24	AACUGCGU CUGAUGA X GAA AGCUUGUU	847
308	CACGCAGUU GGGCACUU	25	AAGUGCCC CUGAUGA X GAA ACUGCGUG	848
316	UGGGCACUU UUGAAGAU	26	AUCUUCAA CUGAUGA X GAA AGUGCCCA	849
317	GGGCACUUU UGAAGAUC	27	GAUCUUCA CUGAUGA X GAA AAGUGCCC	850
318	GGCACUUUU GAAGAUCA	28	UGAUCUUC CUGAUGA X GAA AAAGUGCC	851
325	UUGAAGAUC AUUUUCUC	29	GAGAAAAU CUGAUGA X GAA AUCUUCAA	852
328	AAGAUCAUU UUCUCAGC	30	GCUGAGAA CUGAUGA X GAA AUGAUCUU	853
329	AGAUCAUUU UCUCAGCC	31	GGCUGAGA CUGAUGA X GAA AAUGAUCU	854
330	GAUCAUUUU CUCAGCCU	32	AGGCUGAG CUGAUGA X GAA AAAUGAUC	855
331	AUCAUUUUC UCAGCCUC	33	GAGGCUGA CUGAUGA X GAA AAAAUGAU	856
333	CAUUUUCUC AGCCUCCA	34	UGGAGGCU CUGAUGA X GAA AGAAAAUG	857
339	CUCAGCCUC CAGAGGAU	35	AUCCUCUG CUGAUGA X GAA AGGCUGAG	858
350	GAGGAUGUU CAAUAACU	36	AGUUAUUG CUGAUGA X GAA ACAUCCUC	859
351	AGGAUGUUC AAUAACUG	37	CAGUUAUU CUGAUGA X GAA AACAUCCU	860
355	UGUUCAAUA ACUGUGAG	38	CUCACAGU CUGAUGA X GAA AUUGAACA	861
369	GAGGUGGUC CUUGGGAA	39	UUCCCAAG CUGAUGA X GAA ACCACCUC	862
372	GUGGUCCUU GGGAAUUU	40	AAAUUCCC CUGAUGA X GAA AGGACCAC	863
379	UUGGGAAUU UGGAAAUU	41	AAUUUCCA CUGAUGA X GAA AUUCCCAA	864
380	UGGGAAUUU GGAAAUUA	42	UAAUUUCC CUGAUGA X GAA AAUUCCCA	865

Table III

387	UUGGAAAUU ACCUAUGU	43	ACAUAGGU CUGAUGA X GAA AUUUCCAA	866
388	UGGAAAUUA CCUAUGUG	44	CACAUAGG CUGAUGA X GAA AAUUUCCA	867
392	AAUUACCUA UGUGCAGA	45	UCUGCACA CUGAUGA X GAA AGGUAAUU	868
406	AGAGGAAUU AUGAUCUU	46	AAGAUCAU CUGAUGA X GAA AUUCCUCU	869
407	GAGGAAUUA UGAUCUUU	47	AAAGAUCA CUGAUGA X GAA AAUUCCUC	870
412	AUUAUGAUC UUUCCUUC	48	GAAGGAAA CUGAUGA X GAA AUCAUAAU	871
414	UAUGAUCUU UCCUUCUU	49	AAGAAGGA CUGAUGA X GAA AGAUCAUA	872
415	AUGAUCUUU CCUUCUUA	50	UAAGAAGG CUGAUGA X GAA AAGAUCAU	873
416	UGAUCUUUC CUUCUUAA	51	UUAAGAAG CUGAUGA X GAA AAAGAUCA	874
419	UCUUUCCUU CUUAAAGA	52	UCUUUAAG CUGAUGA X GAA AGGAAAGA	875
420	CUUUCCUUC UUAAAGAC	53	GUCUUUAA CUGAUGA X GAA AAGGAAAG	876
422	UUCCUUCUU AAAGACCA	54	UGGUCUUU CUGAUGA X GAA AGAAGGAA	877
423	UCCUUCUUA AAGACCAU	55	AUGGUCUU CUGAUGA X GAA AAGAAGGA	878
432	AAGACCAUC CAGGAGGU	56	ACCUCCUG CUGAUGA X GAA AUGGUCUU	879
448	UGGCUGGUU AUGUCCUC	57	GAGGACAU CUGAUGA X GAA ACCAGCCA	880
449	GGCUGGUUA UGUCCUCA	58	UGAGGACA CUGAUGA X GAA AACCAGCC	881
453	GGUUAUGUC CUCAUUGC	59	GCAAUGAG CUGAUGA X GAA ACAUAACC	882
456	UAUGUCCUC AUUGCCCU	60	AGGGCAAU CUGAUGA X GAA AGGACAUA	883
459	GUCCUCAUU GCCCUCAA	61	UUGAGGGC CUGAUGA X GAA AUGAGGAC	884
465	AUUGCCCUC AACACAGU	62	ACUGUGUU CUGAUGA X GAA AGGGCAAU	885
483	GAGCGAAUU CCUUUGGA	63	UCCAAAGG CUGAUGA X GAA AUUCGCUC	886
484	AGCGAAUUC CUUUGGAA	64	UUCCAAAG CUGAUGA X GAA AAUUCGCU	887
487	GAAUUCCUU UGGAAAAC	65	GUUUUCCA CUGAUGA X GAA AGGAAUUC	888
488	AAUUCCUUU GGAAAACC	66	GGUUUUCC CUGAUGA X GAA AAGGAAUU	889
504	CUGCAGAUC AUCAGAGG	67	CCUCUGAU CUGAUGA X GAA AUCUGCAG	890
507	CAGAUCAUC AGAGGAAA	68	UUUCCUCU CUGAUGA X GAA AUGAUCUG	891
517	GAGGAAAUA UGUACUAC	69	GUAGUACA CUGAUGA X GAA AUUUCCUC	892
521	AAAUAUGUA CUACGAAA	70	UUUCGUAG CUGAUGA X GAA ACAUAUUU	893
524	UAUGUACUA CGAAAAUU	71	AAUUUUCG CUGAUGA X GAA AGUACAUA	894
532	ACGAAAAUU CCUAUGCC	72	GGCAUAGG CUGAUGA X GAA AUUUUCGU	895
533	CGAAAAUUC CUAUGCCU	73	AGGCAUAG CUGAUGA X GAA AAUUUUCG	896
536	AAAUUCCUA UGCCUUAG	74	CUAAGGCA CUGAUGA X GAA AGGAAUUU	897
542	CUAUGCCUU AGCAGUCU	75	AGACUGCU CUGAUGA X GAA AGGCAUAG	898
543	UAUGCCUUA GCAGUCUU	76	AAGACUGC CUGAUGA X GAA AAGGCAUA	899
549	UUAGCAGUC UUAUCUAA	77	UUAGAUAA CUGAUGA X GAA ACUGCUAA	900
551	AGCAGUCUU AUCUAACU	78	AGUUAGAU CUGAUGA X GAA AGACUGCU	901
552	GCAGUCUUA UCUAACUA	79	UAGUUAGA CUGAUGA X GAA AAGACUGC	902
554	AGUCUUAUC UAACUAUG	80	CAUAGUUA CUGAUGA X GAA AUAAGACU	903
556	UCUUAUCUA ACUAUGAU	81	AUCAUAGU CUGAUGA X GAA AGAUAAGA	904
560	AUCUAACUA UGAUGCAA	82	UUGCAUCA CUGAUGA X GAA AGUUAGAU	905
571	AUGCAAAUA AAACCGGA	83	UCCGGUUU CUGAUGA X GAA AUUUGCAU	906
604	UGAGAAAUU UACAGGAA	84	UUCCUGUA CUGAUGA X GAA AUUUCUCA	907
605	GAGAAAUUU ACAGGAAA	85	UUUCCUGU CUGAUGA X GAA AAUUUCUC	908
606	AGAAAUUUA CAGGAAAU	86	AUUUCCUG CUGAUGA X GAA AAAUUUCU	909
615	CAGGAAAUC CUGCAUGG	87	CCAUGCAG CUGAUGA X GAA AUUUCCUG	910
635	CGUGCGGUU CAGCAACA	88	UGUUGCUG CUGAUGA X GAA ACCGCACG	911
636	GUGCGGUUC AGCAACAA	89	UUGUUGCU CUGAUGA X GAA AACCGCAC	912
672	GAGAGCAUC CAGUGGCG	90	CGCCACUG CUGAUGA X GAA AUGCUCUC	913

Table III

687	CGGGACAUA GUCAGCAG	91	CUGCUGAC CUGAUGA X GAA AUGUCCCG	914
690	GACAUAGUC AGCAGUGA	92	UCACUGCU CUGAUGA X GAA ACUAUGUC	915
701	CAGUGACUU UCUCAGCA	93	UGCUGAGA CUGAUGA X GAA AGUCACUG	916
702	AGUGACUUU CUCAGCAA	94	UUGCUGAG CUGAUGA X GAA AAGUCACU	917
703	GUGACUUUC UCAGCAAC	95	GUUGCUGA CUGAUGA X GAA AAAGUCAC	918
705	GACUUUCUC AGCAACAU	96	AUGUUGCU CUGAUGA X GAA AGAAAGUC	919
716	CAACAUGUC GAUGGACU	97	AGUCCAUC CUGAUGA X GAA ACAUGUUG	920
725	GAUGGACUU CCAGAACC	98	GGUUCUGG CUGAUGA X GAA AGUCCAUC	921
726	AUGGACUUC CAGAACCA	99	UGGUUCUG CUGAUGA X GAA AAGUCCAU	922
760	AGUGUGAUC CAAGCUGU	100	ACAGCUUG CUGAUGA X GAA AUCACACU	923
769	CAAGCUGUC CCAAUGGG	101	CCCAUUGG CUGAUGA X GAA ACAGCUUG	924
825	ACCAAAAUC AUCUGUGC	102	GCACAGAU CUGAUGA X GAA AUUUUGGU	925
828	AAAAUCAUC UGUGCCCA	103	UGGGCACA CUGAUGA X GAA AUGAUUUU	926
845	GCAGUGCUC CGGGCGCU	104	AGCGCCCG CUGAUGA X GAA AGCACUGC	927
866	UGGCAAGUC CCCCAGUG	105	CACUGGGG CUGAUGA X GAA ACUUGCCA	928
936	UGCCUGGUC UGCCGCAA	106	UUGCGGCA CUGAUGA X GAA ACCAGGCA	929
947	CCGCAAAUU CCGAGACG	107	CGUCUCGG CUGAUGA X GAA AUUUGCGG	930
948	CGCAAAUUC CGAGACGA	108	UCGUCUCG CUGAUGA X GAA AAUUUGCG	931
987	CCCCCACUC AUGCUCUA	109	UAGAGCAU CUGAUGA X GAA AGUGGGGG	932
993	CUCAUGCUC UACAACCC	110	GGGUUGUA CUGAUGA X GAA AGCAUGAG	933
995	CAUGCUCUA CAACCCCA	111	UGGGGUUG CUGAUGA X GAA AGAGCAUG	934
1010	CACCACGUA CCAGAUGG	112	CCAUCUGG CUGAUGA X GAA ACGUGGUG	935
1040	GGGCAAAUA CAGCUUUG	113	CAAAGCUG CUGAUGA X GAA AUUUGCCC	936
1046	AUACAGCUU UGGUGCCA	114	UGGCACCA CUGAUGA X GAA AGCUGUAU	937
1047	UACAGCUUU GGUGCCAC	115	GUGGCACC CUGAUGA X GAA AAGCUGUA	938
1072	AGAAGUGUC CCCGUAAU	116	AUUACGGG CUGAUGA X GAA ACACUUCU	939
1078	GUCCCCGUA AUUAUGUG	117	CACAUAAU CUGAUGA X GAA ACGGGGAC	940
1081	CCCGUAAUU AUGUGGUG	118	CACCACAU CUGAUGA X GAA AUUACGGG	941
1082	CCGUAAUUA UGUGGUGA	119	UCACCACA CUGAUGA X GAA AAUUACGG	942
1096	UGACAGAUC ACGGCUCG	120	CGAGCCGU CUGAUGA X GAA AUCUGUCA	943
1103	UCACGGCUC GUGCGUCC	121	GGACGCAC CUGAUGA X GAA AGCCGUGA	944
1110	UCGUGCGUC CGAGCCUG	122	CAGGCUCG CUGAUGA X GAA ACGCACGA	945
1133	CGACAGCUA UGAGAUGG	123	CCAUCUCA CUGAUGA X GAA AGCUGUCG	946
1155	GACGGCGUC CGCAAGUG	124	CACUUGCG CUGAUGA X GAA ACGCCGUC	947
1165	GCAAGUGUA AGAAGUGC	125	GCACUUCU CUGAUGA X GAA ACACUUGC	948
1183	AAGGGCCUU GCCGCAAA	126	UUUGCGGC CUGAUGA X GAA AGGCCCUU	949
1198	AAGUGUGUA ACGGAAUA	127	UAUUCCGU CUGAUGA X GAA ACACACUU	950
1206	AACGGAAUA GGUAUUGG	128	CCAAUACC CUGAUGA X GAA AUUCCGUU	951
1210	GAAUAGGUA UUGGUGAA	129	UUCACCAA CUGAUGA X GAA ACCUAUUC	952
1212	AUAGGUAUU GGUGAAUU	130	AAUUCACC CUGAUGA X GAA AUACCUAU	953
1220	UGGUGAAUU UAAAGACU	131	AGUCUUUA CUGAUGA X GAA AUUCACCA	954
1221	GGUGAAUUU AAAGACUC	132	GAGUCUUU CUGAUGA X GAA AAUUCACC	955
1222	GUGAAUUUA AAGACUCA	133	UGAGUCUU CUGAUGA X GAA AAAUUCAC	956
1229	UAAAGACUC ACUCUCCA	134	UGGAGAGU CUGAUGA X GAA AGUCUUUA	957
1233	GACUCACUC UCCAUAAA	135	UUUAUGGA CUGAUGA X GAA AGUGAGUC	958
1235	CUCACUCUC CAUAAAUG	136	CAUUUAUG CUGAUGA X GAA AGAGUGAG	959
1239	CUCUCCAUA AAUGCUAC	137	GUAGCAUU CUGAUGA X GAA AUGGAGAG	960
1246	UAAAUGCUA CGAAUAUU	138	AAUAUUCG CUGAUGA X GAA AGCAUUUA	961

Table III

1252	CUACGAAUA UUAAACAC	139	GUGUUUAA CUGAUGA X GAA AUUCGUAG	962
1254	ACGAAUAUU AAACACUU	140	AAGUGUUU CUGAUGA X GAA AUAUUCGU	963
1255	CGAAUAUUA AACACUUC	141	GAAGUGUU CUGAUGA X GAA AAUAUUCG	964
1262	UAAACACUU CAAAAACU	142	AGUUUUUG CUGAUGA X GAA AGUGUUUA	965
1263	AAACACUUC AAAAACUG	143	CAGUUUUU CUGAUGA X GAA AAGUGUUU	966
1277	CUGCACCUC CAUCAGUG	144	CACUGAUG CUGAUGA X GAA AGGUGCAG	967
1281	ACCUCCAUC AGUGGCGA	145	UCGCCACU CUGAUGA X GAA AUGGAGGU	968
1291	GUGGCGAUC UCCACAUC	146	GAUGUGGA CUGAUGA X GAA AUCGCCAC	969
1293	GGCGAUCUC CACAUCCU	147	AGGAUGUG CUGAUGA X GAA AGAUCGCC	970
1299	CUCCACAUC CUGCCGGU	148	ACCGGCAG CUGAUGA X GAA AUGUGGAG	971
1313	GGUGGCAUU UAGGGGUG	149	CACCCCUA CUGAUGA X GAA AUGCCACC	972
1314	GUGGCAUUU AGGGGUGA	150	UCACCCCU CUGAUGA X GAA AAUGCCAC	973
1315	UGGCAUUUA GGGGUGAC	151	GUCACCCC CUGAUGA X GAA AAAUGCCA	974
1325	GGGUGACUC CUUCACAC	152	GUGUGAAG CUGAUGA X GAA AGUCACCC	975
1328	UGACUCCUU CACACAUA	153	UAUGUGUG CUGAUGA X GAA AGGAGUCA	976
1329	GACUCCUUC ACACAUAC	154	GUAUGUGU CUGAUGA X GAA AAGGAGUC	977
1336	UCACACAUA CUCCUCCU	155	AGGAGGAG CUGAUGA X GAA AUGUGUGA	978
1339	CACAUACUC CUCCUCUG	156	CAGAGGAG CUGAUGA X GAA AGUAUGUG	979
1342	AUACUCCUC CUCUGGAU	157	AUCCAGAG CUGAUGA X GAA AGGAGUAU	980
1345	CUCCUCCUC UGGAUCCA	158	UGGAUCCA CUGAUGA X GAA AGGAGGAG	981
1351	CUCUGGAUC CACAGGAA	159	UUCCUGUG CUGAUGA X GAA AUCCAGAG	982
1366	AACUGGAUA UUCUGAAA	160	UUUCAGAA CUGAUGA X GAA AUCCAGUU	983
1368	CUGGAUAUU CUGAAAAC	161	GUUUUCAG CUGAUGA X GAA AUAUCCAG	984
1369	UGGAUAUUC UGAAAACC	162	GGUUUUCA CUGAUGA X GAA AAUAUCCA	985
1380	AAAACCGUA AAGGAAAU	163	AUUUCCUU CUGAUGA X GAA ACGGUUUU	986
1389	AAGGAAAUC ACAGGGUU	164	AACCCUGU CUGAUGA X GAA AUUUCCUU	987
1397	CACAGGGUU UUUGCUGA	165	UCAGCAAA CUGAUGA X GAA ACCCUGUG	988
1398	ACAGGGUUU UUGCUGAU	166	AUCAGCAA CUGAUGA X GAA AACCCUGU	989
1399	CAGGGUUUU UGCUGAUU	167	AAUCAGCA CUGAUGA X GAA AAACCCUG	990
1400	AGGGUUUUU GCUGAUUC	168	GAAUCAGC CUGAUGA X GAA AAAACCCU	991
1407	UUGCUGAUU CAGGCUUG	169	CAAGCCUG CUGAUGA X GAA AUCAGCAA	992
1408	UGCUGAUUC AGGCUUGG	170	CCAAGCCU CUGAUGA X GAA AAUCAGCA	993
1414	UUCAGGCUU GGCCUGAA	171	UUCAGGCC CUGAUGA X GAA AGCCUGAA	994
1437	ACGGACCUC CAUGCCUU	172	AAGGCAUG CUGAUGA X GAA AGGUCCGU	995
1445	CCAUGCCUU UGAGAACC	173	GGUUCUCA CUGAUGA X GAA AGGCAUGG	996
1446	CAUGCCUUU GAGAACCU	174	AGGUUCUC CUGAUGA X GAA AAGGCAUG	997
1455	GAGAACCUA GAAAUCAU	175	AUGAUUUC CUGAUGA X GAA AGGUUCUC	998
1461	CUAGAAAUC AUACGCGG	176	CCGCGUAU CUGAUGA X GAA AUUUCUAG	999
1464	GAAAUCAUA CGCGGCAG	177	CUGCCGCG CUGAUGA X GAA AUGAUUUC	1000
1489	AACAUGGUC AGUUUUCU	178	AGAAAACU CUGAUGA X GAA ACCAUGUU	1001
1493	UGGUCAGUU UUCUCUUG	179	CAAGAGAA CUGAUGA X GAA ACUGACCA	1002
1494	GGUCAGUUU UCUCUUGC	180	GCAAGAGA CUGAUGA X GAA AACUGACC	1003
1495	GUCAGUUUU CUCUUGCA	181	UGCAAGAG CUGAUGA X GAA AAACUGAC	1004
1496	UCAGUUUUC UCUUGCAG	182	CUGCAAGA CUGAUGA X GAA AAAACUGA	1005
1498	AGUUUUCUC UUGCAGUC	183	GACUGCAA CUGAUGA X GAA AGAAAACU	1006
1500	UUUUCUCUU GCAGUCGU	184	ACGACUGC CUGAUGA X GAA AGAGAAAA	1007
1506	CUUGCAGUC GUCAGCCU	185	AGGCUGAC CUGAUGA X GAA ACUGCAAG	1008
1509	GCAGUCGUC AGCCUGAA	186	UUCAGGCU CUGAUGA X GAA ACGACUGC	1009

Table III

1521	CUGAACAUA ACAUCCUU	187	AAGGAUGU CUGAUGA X GAA AUGUUCAG	1010
1526	CAUAACAUC CUUGGGAU	188	AUCCCAAG CUGAUGA X GAA AUGUUAUG	1011
1529	AACAUCCUU GGGAUUAC	189	GUAAUCCC CUGAUGA X GAA AGGAUGUU	1012
1535	CUUGGGAUU ACGCUCCC	190	GGGAGCGU CUGAUGA X GAA AUCCCAAG	1013
1536	UUGGGAUUA CGCUCCCU	191	AGGGAGCG CUGAUGA X GAA AAUCCCAA	1014
1541	AUUACGCUC CCUCAAGG	192	CCUUGAGG CUGAUGA X GAA AGCGUAAU	1015
1545	CGCUCCCUC AAGGAGAU	193	AUCUCCUU CUGAUGA X GAA AGGGAGCG	1016
1554	AAGGAGAUA AGUGAUGG	194	CCAUCACU CUGAUGA X GAA AUCUCCUU	1017
1572	GAUGUGAUA AUUUCAGG	195	CCUGAAAU CUGAUGA X GAA AUCACAUC	1018
1575	GUGAUAAUU UCAGGAAA	196	UUUCCUGA CUGAUGA X GAA AUUAUCAC	1019
1576	UGAUAAUUU CAGGAAAC	197	GUUUCCUG CUGAUGA X GAA AAUUAUCA	1020
1577	GAUAAUUUC AGGAAACA	198	UGUUUCCU CUGAUGA X GAA AAAUUAUC	1021
1591	ACAAAAAUU UGUGCUAU	199	AUAGCACA CUGAUGA X GAA AUUUUUGU	1022
1592	CAAAAAUUU GUGCUAUG	200	CAUAGCAC CUGAUGA X GAA AAUUUUUG	1023
1598	UUUGUGCUA UGCAAAUA	201	UAUUUGCA CUGAUGA X GAA AGCACAAA	1024
1606	AUGCAAAUA CAAUAAAC	202	GUUUAUUG CUGAUGA X GAA AUUUGCAU	1025
1611	AAUACAAUA AACUGGAA	203	UUCCAGUU CUGAUGA X GAA AUUGUAUU	1026
1628	AAAACUGUU UGGGACCU	204	AGGUCCCA CUGAUGA X GAA ACAGUUUU	1027
1629	AAACUGUUU GGGACCUC	205	GAGGUCCC CUGAUGA X GAA AACAGUUU	1028
1637	UGGGACCUC CGGUCAGA	206	UCUGACCG CUGAUGA X GAA AGGUCCCA	1029
1642	CCUCCGGUC AGAAAACC	207	GGUUUUCU CUGAUGA X GAA ACCGGAGG	1030
1656	ACCAAAAUU AUAAGCAA	208	UUGCUUAU CUGAUGA X GAA AUUUUGGU	1031
1657	CCAAAAUUA UAAGCAAC	209	GUUGCUUA CUGAUGA X GAA AAUUUUGG	1032
1659	AAAAUUAUA AGCAACAG	210	CUGUUGCU CUGAUGA X GAA AUAAUUUU	1033
1701	GGCCAGGUC UGCCAUGC	211	GCAUGGCA CUGAUGA X GAA ACCUGGCC	1034
1712	CCAUGCCUU GUGCUCCC	212	GGGAGCAC CUGAUGA X GAA AGGCAUGG	1035
1718	CUUGUGCUC CCCCGAGG	213	CCUCGGGG CUGAUGA X GAA AGCACAAG	1036
1758	GACUGCGUC UCUUGCCG	214	CGGCAAGA CUGAUGA x GAA ACGCAGUC	1037
1760	CUGCGUCUC UUGCCGGA	215	UCCGGCAA CUGAUGA X GAA AGACGCAG	1038
1762	GCGUCUCUU GCCGGAAU	216	AUUCCGGC CUGAUGA X GAA AGAGACGC	1039
1773	CGGAAUGUC AGCCGAGG	217	CCUCGGCU CUGAUGA X GAA ACAUUCCG	1040
1809	UGCAAGCUU CUGGAGGG	218	CCCUCCAG CUGAUGA X GAA AGCUUGCA	1041
1810	GCAAGCUUC UGGAGGGU	219	ACCCUCCA CUGAUGA X GAA AAGCUUGC	1042
1832	AAGGGAGUU UGUGGAGA	220	UCUCCACA CUGAUGA X GAA ACUCCCUU	1043
1833	AGGGAGUUU GUGGAGAA	221	UUCUCCAC CUGAUGA X GAA AACUCCCU	1044
1844	GGAGAACUC UGAGUGCA	222	UGCACUCA CUGAUGA X GAA AGUUCUCC	1045
1854	GAGUGCAUA CAGUGCCA	223	UGGCACUG CUGAUGA X GAA AUGCACUC	1046
1879	GCCUGCCUC AGGCCAUG	224	CAUGGCCU CUGAUGA X GAA AGGCAGGC	1047
1893	AUGAACAUC ACCUGCAC	225	GUGCAGGU CUGAUGA X GAA AUGUUCAU	1048
1924	ACAACUGUA UCCAGUGU	226	ACACUGGA CUGAUGA X GAA ACAGUUGU	1049
1926	AACUGUAUC CAGUGUGC	227	GCACACUG CUGAUGA X GAA AUACAGUU	1050
1940	UGCCCACUA CAUUGACG	228	CGUCAAUG CUGAUGA X GAA AGUGGGCA	1051
1944	CACUACAUU GACGGCCC	229	GGGCCGUC CUGAUGA X GAA AUGUAGUG	1052
1962	CACUGCGUC AAGACCUG	230	CAGGUCUU CUGAUGA X GAA ACGCAGUG	1053
1983	GCAGGAGUC AUGGGAGA	231	UCUCCCAU CUGAUGA X GAA ACUCCUGC	1054
2007	ACCCUGGUC UGGAAGUA	232	UACUUCCA CUGAUGA X GAA ACCAGGGU	1055
2015	CUGGAAGUA CGCAGACG	233	CGUCUGCG CUGAUGA X GAA ACUUCCAG	1056
2050	UGUGCCAUC CAAACUGC	234	GCAGUUUG CUGAUGA X GAA AUGGCACA	1057

Table III

2063	CUGCACCUA CGGAUGCA	235	UGCAUCCG CUGAUGA X GAA AGGUGCAG	1058
2083	GGCCAGGUC UUGAAGGC	236	GCCUUCAA CUGAUGA X GAA ACCUGGCC	1059
2085	CCAGGUCUU GAAGGCUG	237	CAGCCUUC CUGAUGA X GAA AGACCUGG	1060
2095	AAGGCUGUC CAACGAAU	238	AUUCGUUG CUGAUGA X GAA ACAGCCUU	1061
2110	AUGGGCCUA AGAUCCCG	239	CGGGAUCU CUGAUGA X GAA AGGCCCAU	1062
2115	CCUAAGAUC CCGUCCAU	240	AUGGACGG CUGAUGA X GAA AUCUUAGG	1063
2120	GAUCCCGUC CAUCGCCA	241	UGGCGAUG CUGAUGA X GAA ACGGGAUC	1064
2124	CCGUCCAUC GCCACUGG	242	CCAGUGGC CUGAUGA X GAA AUGGACGG	1065
2148	GGGGCCCUC CUCUUGCU	243	AGCAAGAG CUGAUGA X GAA AGGGCCCC	1066
2151	GCCCUCCUC UUGCUGCU	244	AGCAGCAA CUGAUGA X GAA AGGAGGGC	1067
2153	CCUCCUCUU GCUGCUGG	245	CCAGCAGC CUGAUGA X GAA AGAGGAGG	1068
2178	CUGGGGAUC GGCCUCUU	246	AAGAGGCC CUGAUGA X GAA AUCCCCAG	1069
2184	AUCGGCCUC UUCAUGCG	247	CGCAUGAA CUGAUGA X GAA AGGCCGAU	1070
2186	CGGCCUCUU CAUGCGAA	248	UUCGCAUG CUGAUGA X GAA AGAGGCCG	1071
2187	GGCCUCUUC AUGCGAAG	249	CUUCGCAU CUGAUGA X GAA AAGAGGCC	1072
2205	CGCCACAUC GUUCGGAA	250	UUCCGAAC CUGAUGA X GAA AUGUGGCG	1073
2208	CACAUCGUU CGGAAGCG	251	CGCUUCCG CUGAUGA X GAA ACGAUGUG	1074
2209	ACAUCGUUC GGAAGCGC	252	GCGCUUCC CUGAUGA X GAA AACGAUGU	1075
2250	AGGGAGCUU GUGGAGCC	253	GGCUCCAC CUGAUGA X GAA AGCUCCCU	1076
2260	UGGAGCCUC UUACACCC	254	GGGUGUAA CUGAUGA X GAA AGGCUCCA	1077
2262	GAGCCUCUU ACACCCAG	255	CUGGGUGU CUGAUGA X GAA AGAGGCUC	1078
2263	AGCCUCUUA CACCCAGU	256	ACUGGGUG CUGAUGA X GAA AAGAGGCU	1079
2281	GAGAAGCUC CCAACCAA	257	UUGGUUGG CUGAUGA X GAA AGCUUCUC	1080
2293	ACCAAGCUC UCUUGAGG	258	CCUCAAGA CUGAUGA X GAA AGCUUGGU	1081
2295	CAAGCUCUC UUGAGGAU	259	AUCCUCAA CUGAUGA X GAA AGAGCUUG	1082
2297	AGCUCUCUU GAGGAUCU	260	AGAUCCUC CUGAUGA X GAA AGAGAGCU	1083
2304	UUGAGGAUC UUGAAGGA	261	UCCUUCAA CUGAUGA X GAA AUCCUCAA	1084
2306	GAGGAUCUU GAAGGAAA	262	UUUCCUUC CUGAUGA X GAA AGAUCCUC	1085
2321	AACUGAAUU CAAAAAGA	263	UCUUUUUG CUGAUGA X GAA AUUCAGUU	1086
2322	ACUGAAUUC AAAAAGAU	264	AUCUUUUU CUGAUGA X GAA AAUUCAGU	1087
2331	AAAAAGAUC AAAGUGCU	265	AGCACUUU CUGAUGA X GAA AUCUUUUU	1088
2345	GCUGGGCUC CGGUGCGU	266	ACGCACCG CUGAUGA X GAA AGCCCAGC	1089
2354	CGGUGCGUU CGGCACGG	267	CCGUGCCG CUGAUGA X GAA ACGCACCG	1090
2355	GGUGCGUUC GGCACGGU	268	ACCGUGCC CUGAUGA X GAA AACGCACC	1091
2366	CACGGUGUA UAAGGGAC	269	GUCCCUUA CUGAUGA X GAA ACACCGUG	1092
2368	CGGUGUAUA AGGGACUC	270	GAGUCCCU CUGAUGA X GAA AUACACCG	1093
2376	AAGGGACUC UGGAUCCC	271	GGGAUCCA CUGAUGA X GAA AGUCCCUU	1094
2382	CUCUGGAUC CCAGAAGG	272	CCUUCUGG CUGAUGA X GAA AUCCAGAG	1095
2400	GAGAAAGUU AAAAUUCC	273	GGAAUUUU CUGAUGA X GAA ACUUUCUC	1096
2401	AGAAAGUUA AAAUUCCC	274	GGGAAUUU CUGAUGA X GAA AACUUUCU	1097
2406	GUUAAAAUU CCCGUCGC	275	GCGACGGG CUGAUGA X GAA AUUUUAAC	1098
2407	UUAAAAUUC CCGUCGCU	276	AGCGACGG CUGAUGA X GAA AAUUUUAA	1099
2412	AUUCCCGUC GCUAUCAA	277	UUGAUAGC CUGAUGA X GAA ACGGGAAU	1100
2416	CCGUCGCUA UCAAGGAA	278	UUCCUUGA CUGAUGA X GAA AGCGACGG	1101
2418	GUCGCUAUC AAGGAAUU	279	AAUUCCUU CUGAUGA X GAA AUAGCGAC	1102
2426	CAAGGAAUU AAGAGAAG	280	CUUCUCUU CUGAUGA X GAA AUUCCUUG	1103
2427	AAGGAAUUA AGAGAAGC	281	GCUUCUCU CUGAUGA X GAA AAUUCCUU	1104
2441	AGCAACAUC UCCGAAAG	282	CUUUCGGA CUGAUGA X GAA AUGUUGCU	1105

Table III

2443	CAACAUCUC CGAAAGCC	283	GGCUUUCG CUGAUGA X GAA AGAUGUUG	1106
2463	AAGGAAAUC CUCGAUGA	284	UCAUCGAG CUGAUGA X GAA AUUUCCUU	1107
2466	GAAAUCCUC GAUGAAGC	285	GCUUCAUC CUGAUGA X GAA AGGAUUUC	1108
2477	UGAAGCCUA CGUGAUGG	286	CCAUCACG CUGAUGA X GAA AGGCUUCA	1109
2526	CUGGGCAUC UGCCUCAC	287	GUGAGGCA CUGAUGA X GAA AUGCCCAG	1110
2532	AUCUGCCUC ACCUCCAC	288	GUGGAGGU CUGAUGA X GAA AGGCAGAU	1111
2537	CCUCACCUC CACCGUGC	289	GCACGGUG CUGAUGA X GAA AGGUGAGG	1112
2550	GUGCAACUC AUCACGCA	290	UGCGUGAU CUGAUGA X GAA AGUUGCAC	1113
2553	CAACUCAUC ACGCAGCU	291	AGCUGCGU CUGAUGA X GAA AUGAGUUG	1114
2562	ACGCAGCUC AUGCCCUU	292	AAGGGCAU CUGAUGA X GAA AGCUGCGU	1115
2570	CAUGCCCUU CGGCUGCC	293	GGCAGCCG CUGAUGA X GAA AGGGCAUG	1116
2571	AUGCCCUUC GGCUGCCU	294	AGGCAGCC CUGAUGA X GAA AAGGGCAU	1117
2580	GGCUGCCUC CUGGACUA	295	UAGUCCAG CUGAUGA X GAA AGGCAGCC	1118
2588	CCUGGACUA UGUCCGGG	296	CCCGGACA CUGAUGA X GAA AGUCCAGG	1119
2592	GACUAUGUC CGGGAACA	297	UGUUCCCG CUGAUGA X GAA ACAUAGUC	1120
2611	AAGACAAUA UUGGCUCC	298	GGAGCCAA CUGAUGA X GAA AUUGUCUU	1121
2613	GACAAUAUU GGCUCCCA	299	UGGGAGCC CUGAUGA X GAA AUAUUGUC	1122
2618	UAUUGGCUC CCAGUACC	300	GGUACUGG CUGAUGA X GAA AGCCAAUA	1123
2624	CUCCCAGUA CCUGCUCA	301	UGAGCAGG CUGAUGA X GAA ACUGGGAG	1124
2631	UACCUGCUC AACUGGUG	302	CACCAGUU CUGAUGA X GAA AGCAGGUA	1125
2649	GUGCAGAUC GCAAAGGG	303	CCCUUUGC CUGAUGA X GAA AUCUGCAC	1126
2666	CAUGAACUA CUUGGAGG	304	CCUCCAAG CUGAUGA X GAA AGUUCAUG	1127
2669	GAACUACUU GGAGGACC	305	GGUCCUCC CUGAUGA X GAA AGUAGUUC	1128
2680	AGGACCGUC GCUUGGUG	306	CACCAAGC CUGAUGA X GAA ACGGUCCU	1129
2684	CCGUCGCUU GGUGCACC	307	GGUGCACC CUGAUGA X GAA AGCGACGG	1130
2715	AGGAACGUA CUGGUGAA	308	UUCACCAG CUGAUGA X GAA ACGUUCCU	1131
2739	CAGCAUGUC AAGAUCAC	309	GUGAUCUU CUGAUGA X GAA ACAUGCUG	1132
2745	GUCAAGAUC ACAGAUUU	310	AAAUCUGU CUGAUGA X GAA AUCUUGAC	1133
2752	UCACAGAUU UUGGGCUG	311	CAGCCCAA CUGAUGA X GAA AUCUGUGA	1134
2753	CACAGAUUU UGGGCUGG	312	CCAGCCCA CUGAUGA X GAA AAUCUGUG	1135
2754	ACAGAUUUU GGGCUGGC	313	GCCAGCCC CUGAUGA X GAA AAAUCUGU	1136
2792	GAAAGAAUA CCAUGCAG	314	CUGCAUGG CUGAUGA X GAA AUUCUUUC	1137
2818	AAGUGCCUA UCAAGUGG	315	CCACUUGA CUGAUGA X GAA AGGCACUU	1138
2820	GUGCCUAUC AAGUGGAU	316	AUCCACUU CUGAUGA X GAA AUAGGCAC	1139
2834	GAUGGCAUU GGAAUCAA	317	UUGAUUCC CUGAUGA X GAA AUGCCAUC	1140
2840	AUUGGAAUC AAUUUUAC	318	GUAAAAUU CUGAUGA X GAA AUUCCAAU	1141
2844	GAAUCAAUU UUACACAG	319	CUGUGUAA CUGAUGA X GAA AUUGAUUC	1142
2845	AAUCAAUUU UACACAGA	320	UCUGUGUA CUGAUGA X GAA AAUUGAUU	1143
2846	AUCAAUUUU ACACAGAA	321	UUCUGUGU CUGAUGA X GAA AAAUUGAU	1144
2847	UCAAUUUUA CACAGAAU	322	AUUCUGUG CUGAUGA X GAA AAAAUUGA	1145
2856	CACAGAAUC UAUACCCA	323	UGGGUAUA CUGAUGA X GAA AUUCUGUG	1146
2858	CAGAAUCUA UACCCACC	324	GGUGGGUA CUGAUGA X GAA AGAUUCUG	1147
2860	GAAUCUAUA CCCACCAG	325	CUGGUGGG CUGAUGA X GAA AUAGAUUC	1148
2877	AGUGAUGUC UGGAGCUA	326	UAGCUCCA CUGAUGA X GAA ACAUCACU	1149
2885	CUGGAGCUA CGGGGUGA	327	UCACCCCG CUGAUGA X GAA AGCUCCAG	1150
2898	GUGACCGUU UGGGAGUU	328	AACUCCCA CUGAUGA X GAA ACGGUCAC	1151
2899	UGACCGUUU GGGAGUUG	329	CAACUCCC CUGAUGA X GAA AACGGUCA	1152
2906	UUGGGAGUU GAUGACCU	330	AGGUCAUC CUGAUGA X GAA ACUCCCAA	1153

Table III

2915	GAUGACCUU UGGAUCCA	331	UGGAUCCA CUGAUGA X GAA AGGUCAUC	1154
2916	AUGACCUUU GGAUCCAA	332	UUGGAUCC CUGAUGA X GAA AAGGUCAU	1155
2921	CUUUGGAUC CAAGCCAU	333	AUGGCUUG CUGAUGA X GAA AUCCAAAG	1156
2930	CAAGCCAUA UGACGGAA	334	UUCCGUCA CUGAUGA X GAA AUGGCUUG	1157
2940	GACGGAAUC CCUGCCAG	335	CUGGCAGG CUGAUGA X GAA AUUCCGUC	1158
2955	AGCGAGAUC UCCUCCAU	336	AUGGAGGA CUGAUGA X GAA AUCUCGCU	1159
2957	CGAGAUCUC CUCCAUCC	337	GGAUGGAG CUGAUGA X GAA AGAUCUCG	1160
2960	GAUCUCCUC CAUCCUGG	338	CCAGGAUG CUGAUGA X GAA AGGAGAUC	1161
2964	UCCUCCAUC CUGGAGAA	339	UUCUCCAG CUGAUGA X GAA AUGGAGGA	1162
2985	GAACGCCUC CCUCAGCC	340	GGCUGAGG CUGAUGA X GAA AGGCGUUC	1163
2989	GCCUCCCUC AGCCACCC	341	GGGUGGCU CUGAUGA X GAA AGGGAGGC	1164
3000	CCACCCAUA UGUACCAU	342	AUGGUACA CUGAUGA X GAA AUGGGUGG	1165
3004	CCAUAUGUA CCAUCGAU	343	AUCGAUGG CUGAUGA X GAA ACAUAUGG	1166
3009	UGUACCAUC GAUGUCUA	344	UAGACAUC CUGAUGA X GAA AUGGUACA	1167
3015	AUCGAUGUC UACAUGAU	345	AUCAUGUA CUGAUGA X GAA ACAUCGAU	1168
3017	CGAUGUCUA CAUGAUCA	346	UGAUCAUG CUGAUGA X GAA AGACAUCG	1169
3024	UACAUGAUC AUGGUCAA	347	UUGACCAU CUGAUGA X GAA AUCAUGUA	1170
3030	AUCAUGGUC AAGUGCUG	348	CAGCACUU CUGAUGA X GAA ACCAUGAU	1171
3045	UGGAUGAUA GACGCAGA	349	UCUGCGUC CUGAUGA X GAA AUCAUCCA	1172
3055	ACGCAGAUA GUCGCCCA	350	UGGGCGAC CUGAUGA X GAA AUCUGCGU	1173
3058	CAGAUAGUC GCCCAAAG	351	CUUUGGGC CUGAUGA X GAA ACUAUCUG	1174
3068	CCCAAAGUU CCGUGAGU	352	ACUCACGG CUGAUGA X GAA ACUUUGGG	1175
3069	CCAAAGUUC CGUGAGUU	353	AACUCACG CUGAUGA X GAA AACUUUGG	1176
3077	CCGUGAGUU GAUCAUCG	354	CGAUGAUC CUGAUGA X GAA ACUCACGG	1177
3081	GAGUUGAUC AUCGAAUU	355	AAUUCGAU CUGAUGA X GAA AUCAACUC	1178
3084	UUGAUCAUC GAAUUCUC	356	GAGAAUUC CUGAUGA X GAA AUGAUCAA	1179
3089	CAUCGAAUU CUCCAAAA	357	UUUUGGAG CUGAUGA X GAA AUUCGAUG	1180
3090	AUCGAAUUC UCCAAAAU	358	AUUUUGGA CUGAUGA X GAA AAUUCGAU	1181
3092	CGAAUUCUC CAAAAUGG	359	CCAUUUUG CUGAUGA X GAA AGAAUUCG	1182
3119	CCAGCGCUA CCUUGUCA	360	UGACAAGG CUGAUGA X GAA AGCGCUGG	1183
3123	CGCUACCUU GUCAUUCA	361	UGAAUGAC CUGAUGA X GAA AGGUAGCG	1184
3126	UACCUUGUC AUUCAGGG	362	CCCUGAAU CUGAUGA X GAA ACAAGGUA	1185
3129	CUUGUCAUU CAGGGGGA	363	UCCCCCUG CUGAUGA X GAA AUGACAAG	1186
3130	UUGUCAUUC AGGGGGAU	364	AUCCCCCU CUGAUGA X GAA AAUGACAA	1187
3151	GAAUGCAUU UGCCAAGU	365	ACUUGGCA CUGAUGA X GAA AUGCAUUC	1188
3152	AAUGCAUUU GCCAAGUC	366	GACUUGGC CUGAUGA X GAA AAUGCAUU	1189
3160	UGCCAAGUC CUACAGAC	367	GUCUGUAG CUGAUGA X GAA ACUUGGCA	1190
3163	CAAGUCCUA CAGACUCC	368	GGAGUCUG CUGAUGA X GAA AGGACUUG	1191
3170	UACAGACUC CAACUUCU	369	AGAAGUUG CUGAUGA X GAA AGUCUGUA	1192
3176	CUCCAACUU CUACCGUG	370	CACGGUAG CUGAUGA X GAA AGUUGGAG	1193
3177	UCCAACUUC UACCGUGC	371	GCACGGUA CUGAUGA X GAA AAGUUGGA	1194
3179	CAACUUCUA CCGUGCCC	372	GGGCACGG CUGAUGA X GAA AGAAGUUG	1195
3233	CGACGAGUA CCUCAUCC	373	GGAUGAGG CUGAUGA X GAA ACUCGUCG	1196
3237	GAGUACCUC AUCCCACA	374	UGUGGGAU CUGAUGA X GAA AGGUACUC	1197
3240	UACCUCAUC CCACAGCA	375	UGCUGUGG CUGAUGA X GAA AUGAGGUA	1198
3254	GCAGGGCUU CUUCAGCA	376	UGCUGAAG CUGAUGA X GAA AGCCCUGC	1199
3255	CAGGGCUUC UUCAGCAG	377	CUGCUGAA CUGAUGA X GAA AAGCCCUG	1200
3257	GGGCUUCUU CAGCAGCC	378	GGCUGCUG CUGAUGA X GAA AGAAGCCC	1201

Table III

3258	GGCUUCUUC AGCAGCCC	379	GGGCUGCU CUGAUGA X GAA AAGAAGCC	1202
3269	CAGCCCCUC CACGUCAC	380	GUGACGUG CUGAUGA X GAA AGGGGCUG	1203
3275	CUCCACGUC ACGGACUC	381	GAGUCCGU CUGAUGA X GAA ACGUGGAG	1204
3283	CACGGACUC CCCUCCUG	382	CAGGAGGG CUGAUGA X GAA AGUCCGUG	1205
3288	ACUCCCCUC CUGAGCUC	383	GAGCUCAG CUGAUGA X GAA AGGGGAGU	1206
3296	CCUGAGCUC UCUGAGUG	384	CACUCAGA CUGAUGA X GAA AGCUCAGG	1207
3298	UGAGCUCUC UGAGUGCA	385	UGCACUCA CUGAUGA X GAA AGAGCUCA	1208
3319	GCAACAAUU CCACCGUG	386	CACGGUGG CUGAUGA X GAA AUUGUUGC	1209
3320	CAACAAUUC CACCGUGG	387	CCACGGUG CUGAUGA X GAA AAUUGUUG	1210
3331	CCGUGGCUU GCAUUGAU	388	AUCAAUGC CUGAUGA X GAA AGCCACGG	1211
3336	GCUUGCAUU GAUAGAAA	389	UUUCUAUC CUGAUGA X GAA AUGCAAGC	1212
3340	GCAUUGAUA GAAAUGGG	390	CCCAUUUC CUGAUGA X GAA AUCAAUGC	1213
3361	AAAGCUGUC CCAUCAAG	391	CUUGAUGG CUGAUGA X GAA ACAGCUUU	1214
3366	UGUCCCAUC AAGGAAGA	392	UCUUCCUU CUGAUGA X GAA AUGGGACA	1215
3380	AGACAGCUU CUUGCAGC	393	GCUGCAAG CUGAUGA X GAA AGCUGUCU	1216
3381	GACAGCUUC UUGCAGCG	394	CGCUGCAA CUGAUGA X GAA AAGCUGUC	1217
3383	CAGCUUCUU GCAGCGAU	395	AUCGCUGC CUGAUGA X GAA AGAAGCUG	1218
3392	GCAGCGAUA CAGCUCAG	396	CUGAGCUG CUGAUGA X GAA AUCGCUGC	1219
3398	AUACAGCUC AGACCCCA	397	UGGGGUCU CUGAUGA X GAA AGCUGUAU	1220
3416	AGGCGCCUU GACUGAGG	398	CCUCAGUC CUGAUGA X GAA AGGCGCCU	1221
3432	GACAGCAUA GACGACAC	399	GUGUCGUC CUGAUGA X GAA AUGCUGUC	1222
3443	CGACACCUU CCUCCCAG	400	CUGGGAGG CUGAUGA X GAA AGGUGUCG	1223
3444	GACACCUUC CUCCCAGU	401	ACUGGGAG CUGAUGA X GAA AAGGUGUC	1224
3447	ACCUUCCUC CCAGUGCC	402	GGCACUGG CUGAUGA X GAA AGGAAGGU	1225
3461	GCCUGAAUA CAUAAACC	403	GGUUUAUG CUGAUGA X GAA AUUCAGGC	1226
3465	GAAUACAUA AACCAGUC	404	GACUGGUU CUGAUGA X GAA AUGUAUUC	1227
3473	AAACCAGUC CGUUCCCA	405	UGGGAACG CUGAUGA X GAA ACUGGUUU	1228
3477	CAGUCCGUU CCCAAAAG	406	CUUUUGGG CUGAUGA X GAA ACGGACUG	1229
3478	AGUCCGUUC CCAAAAGG	407	CCUUUUGG CUGAUGA X GAA AACGGACU	1230
3497	CGCUGGCUC UGUGCAGA	408	UCUGCACA CUGAUGA X GAA AGCCAGCG	1231
3508	UGCAGAAUC CUGUCUAU	409	AUAGACAG CUGAUGA X GAA AUUCUGCA	1232
3513	AAUCCUGUC UAUCACAA	410	UUGUGAUA CUGAUGA X GAA ACAGGAUU	1233
3515	UCCUGUCUA UCACAAUC	411	GAUUGUGA CUGAUGA X GAA AGACAGGA	1234
3517	CUGUCUAUC ACAAUCAG	412	CUGAUUGU CUGAUGA X GAA AUAGACAG	1235
3523	AUCACAAUC AGCCUCUG	413	CAGAGGCU CUGAUGA X GAA AUUGUGAU	1236
3529	AUCAGCCUC UGAACCCC	414	GGGGUUCA CUGAUGA X GAA AGGCUGAU	1237
3560	CCCACACUA CCAGGACC	415	GGUCCUGG CUGAUGA X GAA AGUGUGGG	1238
3599	CCCCGAGUA UCUCAACA	416	UGUUGAGA CUGAUGA X GAA ACUCGGGG	1239
3601	CCGAGUAUC UCAACACU	417	AGUGUUGA CUGAUGA X GAA AUACUCGG	1240
3603	GAGUAUCUC AACACUGU	418	ACAGUGUU CUGAUGA X GAA AGAUACUC	1241
3612	AACACUGUC CAGCCCAC	419	GUGGGCUG CUGAUGA X GAA ACAGUGUU	1242
3627	ACCUGUGUC AACAGCAC	420	GUGCUGUU CUGAUGA X GAA ACACAGGU	1243
3638	CAGCACAUU CGACACCC	421	GGCUGUCG CUGAUGA X GAA AUGUGCUG	1244
3639	AGCACAUUC GACAGCCC	422	GGGCUGUC CUGAUGA X GAA AAUGUGCU	1245
3681	CACCAAAUU AGCCUGGA	423	UCCAGGCU CUGAUGA X GAA AUUUGGUG	1246
3682	ACCAAAUUA GCCUGGAC	424	GUCCAGGC CUGAUGA X GAA AAUUUGGU	1247
3701	CCCUGACUA CCAGCAGG	425	CCUGCUGG CUGAUGA X GAA AGUCAGGG	1248
3713	GCAGGACUU CUUUCCCA	426	UGGGAAAG CUGAUGA X GAA AGUCCUGC	1249

Table III

3714	CAGGACUUC UUUCCCAA	427	UUGGGAAA CUGAUGA X GAA AAGUCCUG	1250
3716	GGACUUCUU UCCCAAGG	428	CCUUGGGA CUGAUGA X GAA AGAAGUCC	1251
3717	GACUUCUUU CCCAAGGA	429	UCCUUGGG CUGAUGA X GAA AAGAAGUC	1252
3718	ACUUCUUUC CCAAGGAA	430	UUCCUUGG CUGAUGA X GAA AAAGAAGU	1253
3744	AAUGGCAUC UUUAAGGG	431	CCCUUAAA CUGAUGA X GAA AUGCCAUU	1254
3746	UGGCAUCUU UAAGGGCU	432	AGCCCUUA CUGAUGA X GAA AGAUGCCA	1255
3747	GGCAUCUUU AAGGGCUC	433	GAGCCCUU CUGAUGA X GAA AAGAUGCC	1256
3748	GCAUCUUUA AGGGCUCC	434	GGAGCCCU CUGAUGA X GAA AAAGAUGC	1257
3755	UAAGGGCUC CACAGCUG	435	CAGCUGUG CUGAUGA X GAA AGCCCUUA	1258
3776	UGCAGAAUA CCUAAGGG	436	CCCUUAGG CUGAUGA X GAA AUUCUGCA	1259
3780	GAAUACCUA AGGGUCGC	437	GCGACCCU CUGAUGA X GAA AGGUAUUC	1260
3786	CUAAGGGUC GCGCCACA	438	UGUGGCGC CUGAUGA X GAA ACCCUUAG	1261
3806	CAGUGAAUU UAUUGGAG	439	CUCCAAUA CUGAUGA X GAA AUUCACUG	1262
3807	AGUGAAUUU AUUGGAGC	440	GCUCCAAU CUGAUGA X GAA AAUUCACU	1263
3808	GUGAAUUUA UUGGAGCA	441	UGCUCCAA CUGAUGA X GAA AAAUUCAC	1264
3810	GAAUUUAUU GGAGCAUG	442	CAUGCUCC CUGAUGA X GAA AUAAAUUC	1265
3831	CGGAGGAUA GUAUGAGC	443	GCUCAUAC CUGAUGA X GAA AUCCUCCG	1266
3834	AGGAUAGUA UGAGCCCU	444	AGGGCUCA CUGAUGA X GAA ACUAUCCU	1267
3843	UGAGCCCUA AAAAUCCA	445	UGGAUUUU CUGAUGA X GAA AGGGCUCA	1268
3849	CUAAAAAUC CAGACUCU	446	AGAGUCUG CUGAUGA X GAA AUUUUUAG	1269
3856	UCCAGACUC UUUCGAUA	447	UAUCGAAA CUGAUGA X GAA AGUCUGGA	1270
3858	CAGACUCUU UCGAUACC	448	GGUAUCGA CUGAUGA X GAA AGAGUCUG	1271
3859	AGACUCUUU CGAUACCC	449	GGGUAUCG CUGAUGA X GAA AAGAGUCU	1272
3860	GACUCUUUC GAUACCCA	450	UGGGUAUC CUGAUGA X GAA AAAGAGUC	1273
3864	CUUUCGAUA CCCAGGAC	451	GUCCUGGG CUGAUGA X GAA AUCGAAAG	1274
3888	CAGCAGGUC CUCCAUCC	452	GGAUGGAG CUGAUGA X GAA ACCUGCUG	1275
3891	CAGGUCCUC CAUCCCAA	453	UUGGGAUG CUGAUGA X GAA AGGACCUG	1276
3895	UCCUCCAUC CCAACAGC	454	GCUGUUGG CUGAUGA X GAA AUGGAGGA	1277
3915	GCCCGCAUU AGCUCUUA	455	UAAGAGCU CUGAUGA X GAA AUGCGGGC	1278
3916	CCCGCAUUA GCUCUUAG	456	CUAAGAGC CUGAUGA X GAA AAUGCGGG	1279
3920	CAUUAGCUC UUAGACCC	457	GGGUCUAA CUGAUGA X GAA AGCUAAUG	1280
3922	UUAGCUCUU AGACCCAC	458	GUGGGUCU CUGAUGA X GAA AGAGCUAA	1281
3923	UAGCUCUUA GACCCACA	459	UGUGGGUC CUGAUGA X GAA AAGAGCUA	1282
3939	AGACUGGUU UUGCAACG	460	CGUUGCAA CUGAUGA X GAA ACCAGUCU	1283
3940	GACUGGUUU UGCAACGU	461	ACGUUGCA CUGAUGA X GAA AACCAGUC	1284
3941	ACUGGUUUU GCAACGUU	462	AACGUUGC CUGAUGA X GAA AAACCAGU	1285
3949	UGCAACGUU UACACCGA	463	UCGGUGUA CUGAUGA X GAA ACGUUGCA	1286
3950	GCAACGUUU ACACCGAC	464	GUCGGUGU CUGAUGA X GAA AACGUUGC	1287
3951	CAACGUUUA CACCGACU	465	AGUCGGUG CUGAUGA X GAA AAACGUUG	1288
3960	CACCGACUA GCCAGGAA	466	UUCCUGGC CUGAUGA X GAA AGUCGGUG	1289
3971	CAGGAAGUA CUUCCACC	467	GGUGGAAG CUGAUGA X GAA ACUUCCUG	1290
3974	GAAGUACUU CCACCUCG	468	CGAGGUGG CUGAUGA X GAA AGUACUUC	1291
3975	AAGUACUUC CACCUCGG	469	CCGAGGUG CUGAUGA X GAA AAGUACUU	1292
3981	UUCCACCUC GGGCACAU	470	AUGUGCCC CUGAUGA X GAA AGGUGGAA	1293
3990	GGGCACAUU UUGGGAAG	471	CUUCCCAA CUGAUGA X GAA AUGUGCCC	1294
3991	GGCACAUUU UGGGAAGU	472	ACUUCCCA CUGAUGA X GAA AAUGUGCC	1295
3992	GCACAUUUU GGGAAGUU	473	AACUUCCC CUGAUGA X GAA AAAUGUGC	1296
4000	UGGGAAGUU GCAUUCCU	474	AGGAAUGC CUGAUGA X GAA ACUUCCCA	1297

Table III

4005	AGUUGCAUU CCUUUGUC	475	GACAAAGG CUGAUGA X GAA AUGCAACU	1298
4006	GUUGCAUUC CUUUGUCU	476	AGACAAAG CUGAUGA X GAA AAUGCAAC	1299
4609	GCAUUCCUU UGUCUUCA	477	UGAAGACA CUGAUGA X GAA AGGAAUGC	1300
4010	CAUUCCUUU GUCUUCAA	478	UUGAAGAC CUGAUGA X GAA AAGGAAUG	1301
4013	UCCUUUGUC UUCAAACU	479	AGUUUGAA CUGAUGA X GAA ACAAAGGA	1302
4015	CUUUGUCUU CAAACUGU	480	ACAGUUUG CUGAUGA X GAA AGACAAAG	1303
4016	UUUGUCUUC AAACUGUG	481	CACAGUUU CUGAUGA X GAA AAGACAAA	1304
4031	UGAAGCAUU UACAGAAA	482	UUUCUGUA CUGAUGA X GAA AUGCUUCA	1305
4032	GAAGCAUUU ACAGAAAC	483	GUUUCUGU CUGAUGA X GAA AAUGCUUC	1306
4033	AAGCAUUUA CAGAAACG	484	CGUUUCUG CUGAUGA X GAA AAAUGCUU	1307
4045	AAACGCAUC CAGCAAGA	485	UCUUGCUG CUGAUGA X GAA AUGCGUUU	1308
4056	GCAAGAAUA UUGUCCCU	486	AGGGACAA CUGAUGA X GAA AUUCUUGC	1309
4058	AAGAAUAUU GUCCCUUU	487	AAAGGGAC CUGAUGA X GAA AUAUUCUU	1310
4061	AAUAUUGUC CCUUUGAG	488	CUCAAAGG CUGAUGA X GAA ACAAUAUU	1311
4065	UUGUCCCUU UGAGCAGA	489	UCUGCUCA CUGAUGA X GAA AGGGACAA	1312
4066	UGUCCCUUU GAGCAGAA	490	UUCUGCUC CUGAUGA X GAA AAGGGACA	1313
4077	GCAGAAAUU UAUCUUUC	491	GAAAGAUA CUGAUGA X GAA AUUUCUGC	1314
4078	CAGAAAUUU AUCUUUCA	492	UGAAAGAU CUGAUGA X GAA AAUUUCUG	1315
4079	AGAAAUUUA UCUUUCAA	493	UUGAAAGA CUGAUGA X GAA AAAUUUCU	1316
4081	AAAUUUAUC UUUCAAAG	494	CUUUGAAA CUGAUGA X GAA AUAAAUUU	1317
4083	AUUUAUCUU UCAAAGAG	495	CUCUUUGA CUGAUGA X GAA AGAUAAAU	1318
4084	UUUAUCUUU CAAAGAGG	496	CCUCUUUG CUGAUGA X GAA AAGAUAAA	1319
4085	UUAUCUUUC AAAGAGGU	497	ACCUCUUU CUGAUGA X GAA AAAGAUAA	1320
4094	AAAGAGGUA UAUUUGAA	498	UUCAAAUA CUGAUGA X GAA ACCUCUUU	1321
4096	AGAGGUAUA UUUGAAAA	499	UUUUCAAA CUGAUGA X GAA AUACCUCU	1322
4098	ACGUAUAUU UGAAAAAA	500	UUUUUUCA CUGAUGA X GAA AUAUACCU	1323
4099	GGUAUAUUU GAAAAAAA	501	UUUUUUUC CUGAUGA X GAA AAUAUACC	1324
4118	AAAAAAGUA UAUGUGAG	502	CUCACAUA CUGAUGA X GAA ACUUUUUU	1325
4120	AAAAGUAUA UGUGAGGA	503	UCCUCACA CUGAUGA X GAA AUACUUUU	1326
4130	GUGAGGAUU UUUAUUGA	504	UCAAUAAA CUGAUGA X GAA AUCCUCAC	1327
4131	UGAGGAUUU UUAUUGAU	505	AUCAAUAA CUGAUGA X GAA AAUCCUCA	1328
4132	GAGGAUUUU UAUUGAUU	506	AAUCAAUA CUGAUGA X GAA AAAUCCUC	1329
4133	AGGAUUUUU AUUGAUUG	507	CAAUCAAU CUGAUGA X GAA AAAAUCCU	1330
4134	GGAUUUUUA UUGAUUGG	508	CCAAUCAA CUGAUGA X GAA AAAAAUCC	1331
4136	AUUUUUAUU GAUUGGGG	509	CCCCAAUC CUGAUGA X GAA AUAAAAAU	1332
4140	UUAUUGAUU GGGGAUCU	510	AGAUCCCC CUGAUGA X GAA AUCAAUAA	1333
4147	UUGGGGAUC UUGGAGUU	511	AACUCCAA CUGAUGA X GAA AUCCCCAA	1334
4149	GGGGAUCUU GGAGUUUU	512	AAAACUCC CUGAUGA X GAA AGAUCCCC	1335
4155	CUUGGAGUU UUUCAUUG	513	CAAUGAAA CUGAUGA X GAA ACUCCAAG	1336
4156	UUGGAGUUU UUCAUUGU	514	ACAAUGAA CUGAUGA X GAA AACUCCAA	1337
4157	UGGAGUUUU UCAUUGUC	515	GACAAUGA CUGAUGA X GAA AAACUCET	1338
4158	GGAGUUUUU CAUUGUCG	516	CGACAAUG CUGAUGA X GAA AAAACUCC	1339
4159	GAGUUUUUC AUUGUCGC	517	GCGACAAU CUGALNA X GAA AAAAACUC	1340
4162	UUUUUCAUU GUCGCUAU	518	AUAGCGAC CUGAUGA X GAA AUGAAAAA	1341
4165	UUCAUUGUC GCUAUUGA	519	UCAAUAGC CUGAUGA X GAA ACAAUGAA	1342
4169	UUGUCGCUA UUGAUUUU	520	AAAAUCAA CUGAUGA X GAA AGCGACAA	1343
4171	GUCGCUAUU GAUUUUUA	521	UAAAAAUC CUGAUGA X GAA AUAGCGAC	1344
4175	CUAUUGAUU UUUACUUC	522	GAAGUAAA CUGAUGA X GAA AUCAAUAG	1345

Table III

4176	UAUUGAUUU UUACUUCA	523	UGAAGUAA CUGAUGA X GAA AAUCAAUA	1346
4177	AUUGAUUUU UACUUCAA	524	UUGAAGUA CUGAUGA X GAA AAAUCAAU	1347
4178	UUGAUUUUU ACUUCAAU	525	AUUGAAGU CUGAUGA X GAA AAAAUCAA	1348
4179	UGAUUUUUA CUUCAAUG	526	CAUUGAAG CUGAUGA X GAA AAAAAUCA	1349
4182	UUUUUACUU CAAUGGGC	527	GCCCAUUG CUGAUGA X GAA AGUAAAAA	1350
4183	UUUUACUUC AAUGGGCU	528	AGCCCAUU CUGAUGA X GAA AAGUAAAA	1351
4192	AAUGGGCUC UUCCAACA	529	UGUUGGAA CUGAUGA X GAA AGCCCAUU	1352
4194	UGGGCUCUU CCAACAAG	530	CUUGUUGG CUGAUGA X GAA AGAGCCCA	1353
4195	GGGCUCUUC CAACAAGG	531	CCUUGUUG CUGAUGA X GAA AAGAGCCC	1354
4212	AAGAAGCUU GCUGGUAG	532	CUACCAGC CUGAUGA X GAA AGCUUCUU	1355
4219	UUGCUGGUA GCACUUGC	533	GCAAGUGC CUGAUGA X GAA ACCAGCAA	1356
4225	GUAGCACUU GCUACCCU	534	AGGGUAGC CUGAUGA X GAA AGUGCUAC	1357
4229	CACUUGCUA CCCUGAGU	535	ACUCAGGG CUGAUGA X GAA AGCAAGUG	1358
4238	CCCUGAGUU CAUCCAGG	536	CCUGGAUG CUGAUGA X GAA ACUCAGGG	1359
4239	CCUGAGUUC AUCCAGGC	537	GCCUGGAU CUGAUGA X GAA AACUCAGG	1360
4242	GAGUUCAUC CAGGCCCA	538	UGGGCCUG CUGAUGA X GAA AUGAACUC	1361
4280	CCACAAGUC UUCCAGAG	539	CUCUGGAA CUGAUGA X GAA ACUUGUGG	1362
4282	ACAAGUCUU CCAGAGGA	540	UCCUCUGG CUGAUGA X GAA AGACUUGU	1363
4283	CAAGUCUUC CAGAGGAU	541	AUCCUCUG CUGAUGA X GAA AAGACUUG	1364
4295	AGGAUGCUU GAUUCCAG	542	CUGGAAUC CUGAUGA X GAA AGCAUCCU	1365
4299	UGCUUGAUU CCAGUGGU	543	ACCACUGG CUGAUGA X GAA AUCAAGCA	1366
4300	GCUUGAUUC CAGUGGUU	544	AACCACUG CUGAUGA X GAA AAUCAAGC	1367
4308	CCAGUGGUU CUGCUUCA	545	UGAAGCAG CUGAUGA X GAA ACCACUGG	1368
4309	CAGUGGUUC UGCUUCAA	546	UUGAAGCA CUGAUGA X GAA AACCACUG	1369
4314	GUUCUGCUU CAAGGCUU	547	AAGCCUUG CUGAUGA X GAA AGCAGAAC	1370
4315	UUCUGCUUC AAGGCUUC	548	GAAGCCUU CUGAUGA X GAA AAGCAGAA	1371
4322	UCAAGGCUU CCACUGCA	549	UGCAGUGG CUGAUGA X GAA AGCCUUGA	1372
4323	CAAGGCUUC CACUGCAA	550	UUGCAGUG CUGAUGA X GAA AAGCCUUG	1373
4338	AAAACACUA AAGAUCCA	551	UGGAUCUU CUGAUGA X GAA AGUGUUUU	1374
4344	CUAAAGAUC CAAGAAGG	552	CCUUCUUG CUGAUGA X GAA AUCUUUAG	1375
4356	GAAGGCCUU CAUGGCCC	553	GGGCCAUG CUGAUGA X GAA AGGCCUUC	1376
4357	AAGGCCUUC AUGGCCCC	554	GGGGCCAU CUGAUGA X GAA AAGGCCUU	1377
4378	GGCCGGAUC GGUACUGU	555	ACAGUACC CUGAUGA X GAA AUCCGGCC	1378
4382	GGAUCGGUA CUGUAUCA	556	UGAUACAG CUGAUGA X GAA ACCGAUCC	1379
4387	GGUACUGUA UCAAGUCA	557	UGACUUGA CUGAUGA X GAA ACAGUACC	1380
4389	UACUGUAUC AAGUCAUG	558	CAUGACUU CUGAUGA X GAA AUACAGUA	1381
4394	UAUCAAGUC AUGGCAGG	559	CCUGCCAU CUGAUGA X GAA ACUUGAUA	1382
4404	UGGCAGGUA CAGUAGGA	560	UCCUACUG CUGAUGA X GAA ACCUGCCA	1383
4409	GGUACAGUA GGAUAAGC	561	GCUUAUCC CUGAUGA X GAA ACUGUACC	1384
4414	AGUAGGAUA AGCCACUC	562	GAGUGGCU CUGAUGA X GAA AUCCUACU	1385
4422	AAGCCACUC UGUCCCUU	563	AAGGGACA CUGAUGA X GAA AGUGGCUU	1386
4426	CACUCUGUC CCUUCCUG	564	CAGGAAGG CUGAUGA X GAA ACAGAGUG	1387
4430	CUGUCCCUU CCUGGGCA	565	UGCCCAGG CUGAUGA X GAA AGGGACAG	1388
4431	UGUCCCUUC CUGGGCAA	566	UUGCCCAG CUGAUGA X GAA AAGGGACA	1389
4462	GGAUGAAUU CUUCCUUA	567	UAAGGAAG CUGAUGA X GAA AUUCAUCC	1390
4463	GAUGAAUUC UUCCUUAG	568	CUAAGGAA CUGAUGA X GAA AAUUCAUC	1391
4465	UGAAUUCUU CCUUAGAC	569	GUCUAAGG CUGAUGA X GAA AGAAUUCA	1392
4466	GAAUUCUUC CUUAGACU	570	AGUCUAAG CUGAUGA X GAA AAGAAUUC	1393

Table III

4469	UUCUUCCUU AGACUUAC	571	GUAAGUCU CUGAUGA X GAA AGGAAGAA	1394
4470	UCUUCCUUA GACUUACU	572	AGUAAGUC CUGAUGA X GAA AAGGAAGA	1395
4475	CUUAGACUU ACUUUUGU	573	ACAAAAGU CUGAUGA X GAA AGUCUAAG	1396
4476	UUAGACUUA CUUUUGUA	574	UACAAAAG CUGAUGA X GAA AAGUCUAA	1397
4479	GACUUACUU UUGUAAAA	575	UUUUACAA CUGAUGA X GAA AGUAAGUC	1398
4480	ACUUACUUU UGUAAAAA	576	UUUUUACA CUGAUGA X GAA AAGUAAGU	1399
4481	CUUACUUUU GUAAAAAU	577	AUUUUUAC CUGAUGA X GAA AAAGUAAG	1400
4484	ACUUUUGUA AAAAUGUC	578	GACAUUUU CUGAUGA X GAA ACAAAAGU	1401
4492	AAAAAUGUC CCCACGGU	579	ACCGUGGG CUGAUGA X GAA ACAUUUUU	1402
4501	CCCACGGUA CUUACUCC	580	GGAGUAAG CUGAUGA X GAA ACCGUGGG	1403
4504	ACGGUACUU ACUCCCCA	581	UGGGGAGU CUGAUGA X GAA AGUACCGU	1404
4505	CGGUACUUA CUCCCCAC	582	GUGGGGAG CUGAUGA X GAA AAGUACCG	1405
4508	UACUUACUC CCCACUGA	583	UCAGUGGG CUGAUGA X GAA AGUAAGUA	1406
4529	CCAGUGGUU UCCAGUCA	584	UGACUGGA CUGAUGA X GAA ACCACUGG	1407
4530	CAGUGGUUU CCAGUCAU	585	AUGACUGG CUGAUGA X GAA AACCACUG	1408
4531	AGUGGUUUC CAGUCAUG	586	CAUGACUG CUGAUGA X GAA AAACCACU	1409
4536	UUUCCAGUC AUGAGCGU	587	ACGCUCAU CUGAUGA X GAA ACUGGAAA	1410
4545	AUGAGCGUU AGACUGAC	588	GUCAGUCU CUGAUGA X GAA ACGCUCAU	1411
4546	UGAGCGUUA GACUGACU	589	AGUCAGUC CUGAUGA X GAA AACGCUCA	1412
4555	GACUGACUU GUUUGUCU	590	AGACAAAC CUGAUGA X GAA AGUCAGUC	1413
4558	UGACUUGUU UGUCUUCC	591	GGAAGACA CUGAUGA X GAA ACAAGUCA	1414
4559	GACUUGUUU GUCUUCCA	592	UGGAAGAC CUGAUGA X GAA AACAAGUC	1415
4562	UUGUUUGUC UUCCAUUC	593	GAAUGGAA CUGAUGA X GAA ACAAACAA	1416
4564	GUUUGUCUU CCAUUCCA	594	UGGAAUGG CUGAUGA X GAA AGACAAAC	1417
4565	UUUGUCUUC CAUUCCAU	595	AUGGAAUG CUGAUGA X GAA AAGACAAA	1418
4569	UCUUCCAUU CCAUUGUU	596	AACAAUGG CUGAUGA X GAA AUGGAAGA	1419
4570	CUUCCAUUC CAUUGUUU	597	AAACAAUG CUGAUGA X GAA AAUGGAAG	1420
4574	CAUUCCAUU GUUUUGAA	598	UUCAAAAC CUGAUGA X GAA AUGGAAUG	1421
4577	UCCAUUGUU UUGAAACU	599	AGUUUCAA CUGAUGA X GAA ACAAUGGA	1422
4578	CCAUUGUUU UGAAACUC	600	GAGUUUCA CUGAUGA X GAA AACAAUGG	1423
4579	CAUUGUUUU GAAACUCA	601	UGAGUUUC CUGAUGA X GAA AAACAAUG	1424
4586	UUGAAACUC AGUAUGCC	602	GGCAUACU CUGAUGA X GAA AGUUUCAA	1425
4590	AACUCAGUA UGCCGCCC	603	GGGCGGCA CUGAUGA X GAA ACUGAGUU	1426
4603	GCCCCUGUC UUGCUGUC	604	GACAGCAA CUGAUGA X GAA ACAGGGGC	1427
4605	CCCUGUCUU GCUGUCAU	605	AUGACAGC CUGAUGA X GAA AGACAGGG	1428
4611	CUUGCUGUC AUGAAAUC	606	GAUUUCAU CUGAUGA X GAA ACAGCAAG	1429
4619	CAUGAAAUC AGCAAGAG	607	CUCUUGCU CUGAUGA X GAA AUUUCAUG	1430
4640	UGACACAUC AAAUAAUA	608	UAUUAUUU CUGAUGA X GAA AUGUGUCA	1431
4645	CAUCAAAUA AUAACUCG	609	CGAGUUAU CUGAUGA X GAA AUUUGAUG	1432
4648	CAAAUAAUA ACUCGGAU	610	AUCCGAGU CUGAUGA X GAA AUUAUUUG	1433
4652	UAAUAACUC GGAUUCCA	611	UGGAAUCC CUGAUGA X GAA AGUUAUUA	1434
4657	ACUCGGAUU CCAGCCCA	612	UGGGCUGG CUGAUGA X GAA AUCCGAGU	1435
4658	CUCGGAUUC CAGCCCAC	613	GUGGGCUG CUGAUGA X GAA AAUCCGAG	1436
4669	GCCCACAUU GGAUUCAU	614	AUGAAUCC CUGAUGA X GAA AUGUGGGC	1437
4674	CAUUGGAUU CAUCAGCA	615	UGCUGAUG CUGAUGA X GAA AUCCAAUG	1438
4675	AUUGGAUUC AUCAGCAU	616	AUGCUGAU CUGAUGA X GAA AAUCCAAU	1439
4678	GGAUUCAUC AGCAUUUG	617	CAAAUGCU CUGAUGA X GAA AUGAAUCC	1440
4684	AUCAGCAUU UGGACCAA	618	UUGGUCCA CUGAUGA X GAA AUGCUGAU	1441

Table III

4685	UCAGCAUUU GGACCAAU	619	AUUGGUCC CUGAUGA X GAA AAUGCUGA	1442
4694	GGACCAAUA GCCCACAG	620	CUGUGGGC CUGAUGA X GAA AUUGGUCC	1443
4718	UGUGGAAUA CCUAAGGA	621	UCCUUAGG CUGAUGA X GAA AUUCCACA	1444
4722	GAAUACCUA AGGAUAAC	622	GUUAUCCU CUGAUGA X GAA AGGUAUUC	1445
4728	CUAAGGAUA ACACCGCU	623	AGCGGUGU CUGAUGA X GAA AUCCUUAG	1446
4737	ACACCGCUU UUGUUCUC	624	GAGAACAA CUGAUGA X GAA AGCGGUGU	1447
4738	CACCGCUUU UGUUCUCG	625	CGAGAACA CUGAUGA X GAA AAGCGGUG	1448
4739	ACCGCUUUU GUUCUCGC	626	GCGAGAAC CUGAUGA X GAA AAAGCGGU	1449
4742	GCUUUUGUU CUCGCAAA	627	UUUGCGAG CUGAUGA X GAA ACAAAAGC	1450
4743	CUUUUGUUC UCGCAAAA	628	UUUUGCGA CUGAUGA X GAA AACAAAAG	1451
4745	UUUGUUCUC GCAAAAAC	629	GUUUUUGC CUGAUGA X GAA AGAACAAA	1452
4756	AAAAACGUA UCUCCUAA	630	UUAGGAGA CUGAUGA X GAA ACGUUUUU	1453
4758	AAACGUAUC UCCUAAUU	631	AAUUAGGA CUGAUGA X GAA AUACGUUU	1454
4760	ACGUAUCUC CUAAUUUG	632	CAAAUUAG CUGAUGA X GAA AGAUACGU	1455
4763	UAUCUCCUA AUUUGAGG	633	CCUCAAAU CUGAUGA X GAA AGGAGAUA	1456
4766	CUCCUAAUU UGAGGCUC	634	GAGCCUCA CUGAUGA X GAA AUUAGGAG	1457
4767	UCCUAAUUU GAGGCUCA	635	UGAGCCUC CUGAUGA X GAA AAUUAGGA	1458
4774	UUGAGGCUC AGAUGAAA	636	UUUCAUCU CUGAUGA X GAA AGCCUCAA	1459
4788	AAAUGCAUC AGGUCCUU	637	AAGGACCU CUGAUGA X GAA AUGCAUUU	1460
4793	CAUCAGGUC CUUUGGGG	638	CCCCAAAG CUGAUGA X GAA ACCUGAUG	1461
4796	CAGGUCCUU UGGGGCAU	639	AUGCCCCA CUGAUGA X GAA AGGACCUG	1462
4797	AGGUCCUUU GGGGCAUA	640	UAUGCCCC CUGAUGA X GAA AAGGACCU	1463
4805	UGGGGCAUA GAUCAGAA	641	UUCUGAUC CUGAUGA X GAA AUGCCCCA	1464
4809	GCAUAGAUC AGAAGACU	642	AGUCUUCU CUGAUGA X GAA AUCUAUGC	1465
4818	AGAAGACUA CAAAAAUG	643	CAUUUUUG CUGAUGA X GAA AGUCUUCU	1466
4835	AAGCUGCUC UGAAAUCU	644	AGAUUUCA CUGAUGA X GAA AGCAGCUU	1467
4842	UCUGAAAUC UCCUUUAG	645	CUAAAGGA CUGAUGA X GAA AUUUCAGA	1468
4844	UGAAAUCUC CUUUAGCC	646	GGCUAAAG CUGAUGA X GAA AGAUUUCA	1469
4847	AAUCUCCUU UAGCCAUC	647	GAUGGCUA CUGAUGA X GAA AGGAGAUU	1470
4848	AUCUCCUUU AGCCAUCA	648	UGAUGGCU CUGAUGA X GAA AAGGAGAU	1471
4849	UCUCCUUUA GCCAUCAC	649	GUGAUGGC CUGAUGA X GAA AAAGGAGA	1472
4855	UUAGCCAUC ACCCCAAC	650	GUUGGGGU CUGAUGA X GAA AUGGCUAA	1473
4874	CCCAAAAUU AGUUUGUG	651	CACAAACU CUGAUGA X GAA AUUUUGGG	1474
4875	CCAAAAUUA GUUUGUGU	652	ACACAAAC CUGAUGA X GAA AAUUUUGG	1475
4878	AAAUUAGUU UGUGUUAC	653	GUAACACA CUGAUGA X GAA ACUAAUUU	1476
4879	AAUUAGUUU GUGUUACU	654	AGUAACAC CUGAUGA X GAA AACUAAUU	1477
4884	GUUUGUGUU ACUUAUGG	655	CCAUAAGU CUGAUGA X GAA ACACAAAC	1478
4885	UUUGUGUUA CUUAUGGA	656	UCCAUAAG CUGAUGA X GAA AACACAAA	1479
4888	GUGUUACUU AUGGAAGA	657	UCUUCCAU CUGAUGA X GAA AGUAACAC	1480
4889	UGUUACUUA UGGAAGAU	658	AUCUUCCA CUGAUGA X GAA AAGUAACA	1481
4898	UGGAAGAUA GUUUUCUC	659	GAGAAAAC CUGAUGA X GAA AUCUUCCA	1482
4901	AAGAUAGUU UUCUCCUU	660	AAGGAGAA CUGAUGA X GAA ACUAUCUU	1483
4902	AGAUAGUUU UCUCCUUU	661	AAAGGAGA CUGAUGA X GAA AACUAUCU	1484
4903	GAUAGUUUU CUCCUUUU	662	AAAAGGAG CUGAUGA X GAA AAACUAUC	1485
4904	AUAGUUUUC UCCUUUUA	663	UAAAAGGA CUGAUGA X GAA AAAACUAU	1486
4906	AGUUUUCUC CUUUUACU	664	AGUAAAAG CUGAUGA X GAA AGAAAACU	1487
4909	UUUCUCCUU UUACUUCA	665	UGAAGUAA CUGAUGA X GAA AGGAGAAA	1488
4910	UUCUCCUUU UACUUCAC	666	GUGAAGUA CUGAUGA X GAA AAGGAGAA	1489

Table III

4911	UCUCCUUUU ACUUCACU	667	AGUGAAGU CUGAUGA X GAA AAAGGAGA	1490
4912	CUCCUUUUA CUUCACUU	668	AAGUGAAG CUGAUGA X GAA AAAAGGAG	1491
4915	CUUUUACUU CACUUCAA	669	UUGAAGUG CUGAUGA X GAA AGUAAAAG	1492
4916	UUUUACUUC ACUUCAAA	670	UUUGAAGU CUGAUGA X GAA AAGUAAAA	1493
4920	ACUUCACUU CAAAAGCU	671	AGCUUUUG CUGAUGA X GAA AGUGAAGU	1494
4921	CUUCACUUC AAAAGCUU	672	AAGCUUUU CUGAUGA X GAA AAGUGAAG	1495
4929	CAAAAGCUU UUUACUCA	673	UGAGUAAA CUGAUGA X GAA AGCUUUUG	1496
4930	AAAAGCUUU UUACUCAA	674	UUGAGUAA CUGAUGA X GAA AAGCUUUU	1497
4931	AAAGCUUUU UACUCAAA	675	UUUGAGUA CUGAUGA X GAA AAAGCUUU	1498
4932	AAGCUUUUU ACUCAAAG	676	CUUUGAGU CUGAUGA X GAA AAAAGCUU	1499
4933	AGCUUUUUA CUCAAAGA	677	UCUUUGAG CUGAUGA X GAA AAAAAGCU	1500
4936	UUUUUACUC AAAGAGUA	678	UACUCUUU CUGAUGA X GAA AGUAAAAA	1501
4944	CAAAGAGUA UAUGUUCC	679	GGAACAUA CUGAUGA X GAA ACUCUUUG	1502
4946	AAGAGUAUA UGUUCCCU	680	AGGGAACA CUGAUGA X GAA AUACUCUU	1503
4950	GUAUAUGUU CCCUCCAG	681	CUGGAGGG CUGAUGA X GAA ACAUAUAC	1504
4951	UAUAUGUUC CCUCCAGG	682	CCUGGAGG CUGAUGA X GAA AACAUAUA	1505
4955	UGUUCCCUC CAGGUCAG	683	CUGACCUG CUGAUGA X GAA AGGGAACA	1506
4961	CUCCAGGUC AGCUGCCC	684	GGGCAGCU CUGAUGA X GAA ACCUGGAG	1507
4981	AACCCCCUC CUUACGCU	685	AGCGUAAG CUGAUGA X GAA AGGGGGUU	1508
4984	CCCCUCCUU ACGCUUUG	686	CAAAGCGU CUGAUGA X GAA AGGAGGGG	1509
4985	CCCUCCUUA CGCUUUGU	687	ACAAAGCG CUGAUGA X GAA AAGGAGGG	1510
4990	CUUACGCUU UGUCACAC	688	GUGUGACA CUGAUGA X GAA AGCGUAAG	1511
4991	UUACGCUUU GUCACACA	689	UGUGUGAC CUGAUGA X GAA AAGCGUAA	1512
4994	CGCUUUGUC ACACAAAA	690	UUUUGUGU CUGAUGA X GAA ACAAAGCG	1513
5008	AAAAGUGUC UCUGCCUU	691	AAGGCAGA CUGAUGA X GAA ACACUUUU	1514
5010	AAGUGUCUC UGCCUUGA	692	UCAAGGCA CUGAUGA X GAA AGACACUU	1515
5016	CUCUGCCUU GAGUCAUC	693	GAUGACUC CUGAUGA X GAA AGGCAGAG	1516
5021	CCUUGAGUC AUCUAUUC	694	GAAUAGAU CUGAUGA X GAA ACUCAAGG	1517
5024	UGAGUCAUC UAUUCAAG	695	CUUGAAUA CUGAUGA X GAA AUGACUCA	1518
5026	AGUCAUCUA UUCAAGCA	696	UGCUUGAA CUGAUGA X GAA AGAUGACU	1519
5028	UCAUCUAUU CAAGCACU	697	AGUGCUUG CUGAUGA X GAA AUAGAUGA	1520
5029	CAUCUAUUC AAGCACUU	698	AAGUGCUU CUGAUGA X GAA AAUAGAUG	1521
5037	CAAGCACUU ACAGCUCU	699	AGAGCUGU CUGAUGA X GAA AGUGCUUG	1522
5038	AAGCACUUA CAGCUCUG	700	CAGAGCUG CUGAUGA X GAA AAGUGCUU	1523
5044	UUACAGCUC UGGCCACA	701	UGUGGCCA CUGAUGA X GAA AGCUGUAA	1524
5062	CAGGGCAUU UUACAGGU	702	ACCUGUAA CUGAUGA X GAA AUGCCCUG	1525
5063	AGGGCAUUU UACAGGUG	703	CACCUGUA CUGAUGA X GAA AAUGCCCU	1526
5064	GGGCAUUUU ACAGGUGC	704	GCACCUGU CUGAUGA X GAA AAAUGCCC	1527
5065	GGCAUUUUA CAGGUGCG	705	CGCACCUG CUGAUGA X GAA AAAAUGCC	1528
5083	AUGACAGUA GCAUUAUG	706	CAUAAUGC CUGAUGA X GAA ACUGUCAU	1529
5088	AGUAGCAUU AUGAGUAG	707	CUACUCAU CUGAUGA X GAA AUGCUACU	1530
5089	GUAGCAUUA UGAGUAGU	708	ACUACUCA CUGAUGA X GAA AAUGCUAC	1531
5095	UUAUGAGUA GUGUGAAU	709	AUUCACAC CUGAUGA X GAA ACUCAUAA	1532
5104	GUGUGAAUU CAGGUAGU	710	ACUACCUG CUGAUGA X GAA AUUCACAC	1533
5105	UGUGAAUUC AGGUAGUA	711	UACUACCU CUGAUGA X GAA AAUUCACA	1534
5110	AUUCAGGUA GUAAAUAU	712	AUAUUUAC CUGAUGA X GAA ACCUGAAU	1535
5113	CAGGUAGUA AAUAUGAA	713	UUCAUAUU CUGAUGA X GAA ACUACCUG	1536
5117	UAGUAAAUA UGAAACUA	714	UAGUUUCA CUGAUGA X GAA AUUUACUA	1537

Table III

5125	AUGAAACUA GGGUUUGA	715	UCAAACCC CUGAUGA X GAA AGUUUCAU	1538
5130	ACUAGGGUU UGAAAUUG	716	CAAUUUCA CUGAUGA X GAA ACCCUAGU	1539
5131	CUAGGGUUU GAAAUUGA	717	UCAAUUUC CUGAUGA X GAA AACCCUAG	1540
5137	UUUGAAAUU GAUAAUGC	718	GCAUUAUC CUGAUGA X GAA AUUUCAAA	1541
5141	AAAUUGAUA AUGCUUUC	719	GAAAGCAU CUGAUGA X GAA AUCAAUUU	1542
5147	AUAAUGCUU UCACAACA	720	UGUUGUGA CUGAUGA X GAA AGCAUUAU	1543
7775148	UAAUGCUUU CACAACAU	721	AUGUUGUG CUGAUGA X GAA AAGCAUUA	1544
5149	AAUGCUUUC ACAACAUU	722	AAUGUUGU CUGAUGA X GAA AAAGCAUU	1545
5157	CACAACAUU UGCAGAUG	723	CAUCUGCA CUGAUGA X GAA AUGUUGUG	1546
5158	ACAACAUUU GCAGAUGU	724	ACAUCUGC CUGAUGA X GAA AAUGUUGU	1547
5167	GCAGAUGUU UUAGAAGG	725	CCUUCUAA CUGAUGA X GAA ACAUCUGC	1548
5168	CAGAUGUUU UAGAAGGA	726	UCCUUCUA CUGAUGA X GAA AACAUCUG	1549
5169	AGAUGUUUU AGAAGGAA	727	UUCCUUCU CUGAUGA X GAA AAACAUCU	1550
5170	GAUGUUUUA GAAGGAAA	728	UUUCCUUC CUGAUGA X GAA AAAACAUC	1551
5184	AAAAAAGUU CCUUCCUA	729	UAGGAAGG CUGAUGA X GAA ACUUUUUU	1552
5185	AAAAAGUUC CUUCCUAA	730	UUAGGAAG CUGAUGA X GAA AACUUUUU	1553
5188	AAGUUCCUU CCUAAAAU	731	AUUUUAGG CUGAUGA X GAA AGGAACUU	1554
5189	AGUUCCUUC CUAAAAUA	732	UAUUUUAG CUGAUGA X GAA AAGGAACU	1555
5192	UCCUUCCUA AAAUAAUU	733	AAUUAUUU CUGAUGA X GAA AGGAAGGA	1556
5197	CCUAAAAUA AUUUCUCU	734	AGAGAAAU CUGAUGA X GAA AUUUUAGG	1557
5200	AAAAUAAUU UCUCUACA	735	UGUAGAGA CUGAUGA X GAA AUUAUUUU	1558
5201	AAAUAAUUU CUCUACAA	736	UUGUAGAG CUGAUGA X GAA AAUUAUUU	1559
5202	AAUAAUUUC UCUACAAU	737	AUUGUAGA CUGAUGA X GAA AAAUUAUU	1560
5204	UAAUUUCUC UACAAUUG	738	CAAUUGUA CUGAUGA X GAA AGAAAUUA	1561
5206	AUUUCUCUA CAAUUGGA	739	UCCAAUUG CUGAUGA X GAA AGAGAAAU	1562
5211	UCUACAAUU GGAAGAUU	740	AAUCUUCC CUGAUGA X GAA AUUGUAGA	1563
5219	UGGAAGAUU GGAAGAUU	741	AAUCUUCC CUGAUGA X GAA AUCUUCCA	1564
5227	UGGAAGAUU CAGCUAGU	742	ACUAGCUG CUGAUGA X GAA AUCUUCCA	1565
5228	GGAAGAUUC AGCUAGUU	743	AACUAGCU CUGAUGA X GAA AAUCUUCC	1566
5233	AUUCAGCUA GUUAGGAG	744	CUCCUAAC CUGAUGA X GAA AGCUGAAU	1567
5236	CAGCUAGUU AGGAGCCC	745	GGGCUCCU CUGAUGA X GAA ACUAGCUG	1568
5237	AGCUAGUUA GGAGCCCA	746	UGGGCUCC CUGAUGA X GAA AACUAGCU	1569
5247	GAGCCCAUU UUUUCCUA	747	UAGGAAAA CUGAUGA X GAA AUGGGCUC	1570
5248	AGCCCAUUU UUUCCUAA	748	UUAGGAAA CUGAUGA X GAA AAUGGGCU	1571
5249	GCCCAUUUU UUCCUAAU	749	AUUAGGAA CUGAUGA X GAA AAAUGGGC	1572
5250	CCCAUUUUU UCCUAAUC	750	GAUUAGGA CUGAUGA X GAA AAAAUGGG	1573
5251	CCAUUUUUU CCUAAUCU	751	AGAUUAGG CUGAUGA X GAA AAAAAUGG	1574
5252	CAUUUUUUC CUAAUCUG	752	CAGAUUAG CUGAUGA X GAA AAAAAAUG	1575
5255	UUUUUCCUA AUCUGUGU	753	ACACAGAU CUGAUGA X GAA AGGAAAAA	1576
5258	UUCCUAAUC UGUGUGUG	754	CACACACA CUGAUGA X GAA AUUAGGAA	1577
5273	UGCCCUGUA ACCUGACU	755	AGUCAGGU CUGAUGA X GAA ACAGGGCA	1578
5285	UGACUGGUU AACAGCAG	756	CUGCUGUU CUGAUGA X GAA ACCAGUCA	1579
5286	GACUGGUUA ACAGCAGU	757	ACUGCUGU CUGAUGA X GAA AACCAGUC	1580
5295	ACAGCAGUC CUUUGUAA	758	UUACAAAG CUGAUGA X GAA ACUGCUGU	1581
5298	GCAGUCCUU UGUAAACA	759	UGUUUACA CUGAUGA X GAA AGGACUGC	1582
5299	CAGUCCUUU GUAAACAG	760	CUGUUUAC CUGAUGA X GAA AAGGACUG	1583
5302	UCCUUUGUA AACAGUGU	761	ACACUGUU CUGAUGA X GAA ACAAAGGA	1584
5311	AACAGUGUU UUAAACUC	762	GAGUUUAA CUGAUGA X GAA ACACUGUU	1585

Table III

5312	ACAGUGUUU UAAACUCU	763	AGAGUUUA CUGAUGA X GAA AACACUGU	1586
5313	CAGUGUUUU AAACUCUC	764	GAGAGUUU CUGAUGA X GAA AAACACUG	1587
5314	AGUGUUUUA AACUCUCC	765	GGAGAGUU CUGAUGA X GAA AAAACACU	1588
5319	UUUAAACUC UCCUAGUC	766	GACUAGGA CUGAUGA X GAA AGUUUAAA	1589
5321	UAAACUCUC CUAGUCAA	767	UUGACUAG CUGAUGA X GAA AGAGUUUA	1590
5324	ACUCUCCUA GUCAAUAU	768	AUAUUGAC CUGAUGA X GAA AGGAGAGU	1591
5327	CUCCUAGUC AAUAUCCA	769	UGGAUAUU CUGAUGA X GAA ACUAGGAG	1592
5331	UAGUCAAUA UCCACCCC	770	GGGGUGGA CUGAUGA X GAA AUUGACUA	1593
5333	GUCAAUAUC CACCCCAU	771	AUGGGGUG CUGAUGA X GAA AUAUUGAC	1594
5342	CACCCCAUC CAAUUUAU	772	AUAAAUUG CUGAUGA X GAA AUGGGGUG	1595
5347	CAUCCAAUU UAUCAAGG	773	CCUUGAUA CUGAUGA X GAA AUUGGAUG	1596
5348	AUCCAAUUU AUCAAGGA	774	UCCUUGAU CUGAUGA X GAA AAUUGGAU	1597
5349	UCCAAUUUA UCAAGGAA	775	UUCCUUGA CUGAUGA X GAA AAAUUGGA	1598
5351	CAAUUUAUC AAGGAAGA	776	UCUUCCUU CUGAUGA X GAA AUAAAUUG	1599
5366	GAAAUGGUU CAGAAAAU	777	AUUUUCUG CUGAUGA X GAA ACCAUUUC	1600
5367	AAAUGGUUC AGAAAAUA	778	UAUUUUCU CUGAUGA X GAA AACCAUUU	1601
5375	CAGAAAAUA UUUUCAGC	779	GCUGAAAA CUGAUGA X GAA AUUUUCUG	1602
5377	GAAAAUAUU UUCAGCCU	780	AGGCUGAA CUGAUGA X GAA AUAUUUUC	1603
5378	AAAAUAUUU UCAGCCUA	781	UAGGCUGA CUGAUGA X GAA AAUAUUUU	1604
5379	AAAUAUUUU CAGCCUAC	782	GUAGGCUG CUGAUGA X GAA AAAUAUUU	1605
5380	AAUAUUUUC AGCCUACA	783	UGUAGGCU CUGAUGA X GAA AAAAUAUU	1606
5386	UUCAGCCUA CAGUUAUG	784	CAUAACUG CUGAUGA X GAA AGGCUGAA	1607
5391	CCUACAGUU AUGUUCAG	785	CUGAACAU CUGAUGA X GAA ACUGUAGG	1608
5392	CUACAGUUA UGUUCAGU	786	ACUGAACA CUGAUGA X GAA AACUGUAG	1609
5396	AGUUAUGUU CAGUCACA	787	UGUGACUG CUGAUGA X GAA ACAUAACU	1610
5397	GUUAUGUUC AGUCACAC	788	GUGUGACU CUGAUGA X GAA AACAUAAC	1611
5401	UGUUCAGUC ACACACAC	789	GUGUGUGU CUGAUGA X GAA ACUGAACA	1612
5412	ACACACAUA CAAAAUGU	790	ACAUUUUG CUGAUGA X GAA AUGUGUGU	1613
5421	CAAAAUGUU CCUUUUGC	791	GCAAAAGG CUGAUGA X GAA ACAUUUUG	1614
5422	AAAAUGUUC CUUUUGCU	792	AGCAAAAG CUGAUGA X GAA AACAUUUU	1615
5425	AUGUUCCUU UUGCUUUU	793	AAAAGCAA CUGAUGA X GAA AGGAACAU	1616
5426	UGUUCCUUU UGCUUUUA	794	UAAAAGCA CUGAUGA X GAA AAGGAACA	1617
5427	GUUCCUUUU GCUUUUAA	795	UUAAAAGC CUGAUGA X GAA AAAGGAAC	1618
5431	CUUUUGCUU UUAAAGUA	796	UACUUUAA CUGAUGA X GAA AGCAAAAG	1619
5432	UUUUGCUUU UAAAGUAA	797	UUACUUUA CUGAUGA X GAA AAGCAAAA	1620
5433	UUUGCUUUU AAAGUAAU	798	AUUACUUU CUGAUGA X GAA AAAGCAAA	1621
5434	UUGCUUUUA AAGUAAUU	799	AAUUACUU CUGAUGA X GAA AAAAGCAA	1622
5439	UUUAAAGUA AUUUUUGA	800	UCAAAAAU CUGAUGA X GAA ACUUUAAA	1623
5442	AAAGUAAUU UUUGACUC	801	GAGUCAAA CUGAUGA X GAA AUUACUUU	1624
5443	AAGUAAUUU UUGACUCC	802	GGAGUCAA CUGAUGA X GAA AAUUACUU	1625
5444	AGUAAUUUU UGACUCCC	803	GGGAGUCA CUGAUGA X GAA AAAUUACU	1626
5445	GUAAUUUUU GACUCCCA	804	UGGGAGUC CUGAUGA X GAA AAAAUUAC	1627
5450	UUUUGACUC CCAGAUCA	805	UGAUCUGG CUGAUGA X GAA AGUCAAAA	1628
5457	UCCCAGAUC AGUCAGAG	806	CUCUGACU CUGAUGA X GAA AUCUGGGA	1629
5461	AGAUCAGUC AGAGCCCC	807	GGGGCUCU CUGAUGA X GAA ACUGAUCU	1630
5471	GAGCCCCUA CAGCAUUG	808	CAAUGCUG CUGAUGA X GAA AGGGGCUC	1631
5478	UACAGCAUU GUUAAGAA	809	UUCUUAAC CUGAUGA X GAA AUGCUGUA	1632
5481	AGCAUUGUU AAGAAAGU	810	ACUUUCUU CUGAUGA X GAA ACAAUGCU	1633

Table III

5482	GCAUUGUUA AGAAAGUA	811	UACUUUCU CUGAUGA X GAA AACAAUGC	1634
5490	AAGAAAGUA UUUGAUUU	812	AAAUCAAA CUGAUGA X GAA ACUUUCUU	1635
5492	GAAAGUAUU UGAUUUUU	813	AAAAAUCA CUGAUGA X GAA AUACUUUC	1636
5493	AAAGUAUUU GAUUUUUG	814	CAAAAAUC CUGAUGA X CAA AAUACUUU	1637
5497	UAUUUGAUU UUUGUCUC	815	GAGACAAA CUGAUGA X GAA AUCAAAUA	1638
5498	AUUUGAUUU UUGUCUCA	816	UGAGACAA CUGAUGA X GAA AAUCAAAU	1639
5499	UUUGAUUUU UGUCUCAA	817	UUGAGACA CUGAUGA X GAA AAAUCAAA	1640
5500	UUGAUUUUU GUCUCAAU	818	AUUGAGAC CUGAUGA X GAA AAAAUCAA	1641
5503	AUUUUUGUC UCAAUGAA	819	UUCAUUGA CUGAUGA X GAA ACAAAAAU	1642
5505	UUUUGUCUC AAUGAAAA	820	UUUUCAUU CUGAUGA X GAA AGACAAAA	1643
5515	AUGAAAAUA AAACUAUA	821	UAUAGUUU CUGAUGA X GAA AUUUUCAU	1644
5521	AUAAAACUA UAUUCAUU	822	AAUGAAUA CUGAUGA X GAA AGUUUUAU	1645
5523	AAAACUAUA UUCAUUUC	823	GAAAUGAA CUGAUGA X GAA AUAGUUUU	1646

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20 3252). The length of stem II may be ≥2 base-pairs.

Table IV

TABLE IV: Human EGF-R Hairpin Ribozyme and Target Sequence

nt. Position	Ribosyme	Seq. ID NOs.	Substrate	Seq. ID NOs.
38	GGCGGC AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1647	GCGCC GCC GCCGCC	1759
41	CUGGGC AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1648	CCGCC GCC GCCCAG	1760
44	GGUCUG AGAA GCGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1649	CCGCC GCC CAGACC	1761
49	CGUCCG AGAA GGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1650	GCCCA GAC CGGACG	1762
54	CCUGUC AGAA GGUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1651	GACCG GAC GACAGG	1763
80	GACUCG AGAA GACG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1652	CGUCC GCC CGAGUC	1764
92	CGGCGA AGAA GGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1653	UCCCC GCC UCGCCG	1765
125	UCAGGG AGAA GUGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1654	GCACG GCC CCCUGA	1766
132	GACGGA AGAA GGGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1655	CCCCU GAC UCCGUC	1767
138	AUACUG AGAA GAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1656	ACUCC GUC CAGUAU	1768
204	UGCCCC AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1657	GGACG GCC GGGGCA	1769
227	GCAGCC AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1658	GCGCU GCU GGCUGC	1770
241	UCGCCG AGAA GAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1659	GCUCU GCC CGGCGA	1771
305	GUGCCC AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1660	ACGCA GUU GGGCAC	1772
334	UCUGGA AGAA GAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1661	UCUCA GCC UCCAGA	1773
500	CUGAUG AGAA GCAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1662	CUGCA GAU CAUCAG	1774
546	AGAUAA AGAA GCUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1663	UAGCA GUC UUAUCU	1775
577	CCUUCA AGAA GGUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1664	AACCG GAC UGAAGG	1776
590	CUCAUG AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1665	GAGCU GCC CAUGAG	1777
632	UUGCUG AGAA GCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1666	GUGCG GUU CAGCAA	1778
648	GCACAG AGAA GGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1667	ACCCU GCC CUGUGC	1779
742	UUUGGC AGAA GCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1668	GGGCA GCU GCCAAA	1780
766	CAUUGG AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1669	AAGCU GUC CCAAUG	1781

Table IV

781	CACCCC AGAA GCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1670	GAGCU GCU GGGGUG	1782
815	AUUUUG AGAA GUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1671	AAACU GAC CAAAAU	1783
853	UGCCAC AGAA GCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1672	GCGCU GCC GUGGCA	1784
877	UGUGGC AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1673	UGACU GCU GCCACA	1785
928	AGACCA AGAA GUCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1674	CGACU GCC UGGUCU	1786
937	AUUUGC AGAA GACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1675	GGUCU GCC GCAAAU	1787
976	GUGGGG AGAA GGUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1676	CACCU GCC CCCCAC	1788
1013	ACAUCC AGAA GGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1677	UACCA GAU GGAUGU	1789
1042	CACCAA AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1678	AUACA GCU UUGGUG	1790
1092	GCCGUG AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1679	UGACA GAU CACGGC	1791
1099	CGCACG AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1680	UCACG GCU CGUGCG	1792
1301	GCCACC AGAA GGAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1681	AUCCU GCC GGUGGC	1793
1403	GCCUGA AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1682	UUGCU GAU UCAGGC	1794
1431	AUGGAG AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1683	GGACG GAC CUCCAU	1795
1490	AGAGAA AGAA GACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1684	GGUCA GUU UUCUCU	1796
1503	GCUGAC AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1685	UUGCA GUC GUCAGC	1797
1510	UGUUCA AGAA GACG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1686	CGUCA GCC UGAACA	1798
1625	GUCCCA AGAA GUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1687	AAACU GUU UGGGAC	1799
1678	CCUUGC AGAA GUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1688	AAACA GCU GCAAGG	1800
1729	GGCCCC AGAA GCCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1689	GGGCU GCU GGGGCC	1801
1774	UGCCUC AGAA GACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1690	UGUCA GCC GAGGCA	1802
1874	GCCUGA AGAA GGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1691	UGCCU GCC UCAGGC	1803
1948	AGUGGG AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1692	UGACG GCC CCCACU	1804
1969	CUGCCG AGAA GGUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1693	GACCU GCC CGGCAG	1805
2019	GCCGGC AGAA GCGU	1694	ACGCA GAC GCCGGC	1806

Table IV

	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA			
2065	CAGUGC AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1695	CUACG GAU GCACUG	1807
2092	UCGUUG AGAA GCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1696	AGGCU GUC CAACGA	1808
2117	GCGAUG AGAA GGAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1697	AUCCC GUC CAUCGC	1809
2156	ACCACC AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1698	UUGCU GCU GGUGGU	1810
2179	UGAAGA AGAA GAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1699	GAUCG GCC UCUUCA	1811
2231	UCCUGC AGAA GCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1700	AGGCU GCU GCAGGA	1812
2409	GAUAGC AGAA GGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1701	UUCCC GUC GCUAUC	1813
2512	CCAGCA AGAA GCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1702	GUGCC GCC UGCUGG	1814
2516	AUGCCC AGAA GGCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1703	CGCCU GCU GGGCAU	1815
2527	AGGUGA AGAA GAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1704	CAUCU GCC UCACCU	1816
2558	GGCAUG AGAA GCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1705	ACGCA GCU CAUGCC	1817
2572	GGAGGC AGAA GAAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1706	CUUCG GCU GCCUCC	1818
2575	CCAGGA AGAA GCCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1707	CGGCU GCC UCCUGG	1819
2627	CAGUUG AGAA GGUA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1708	UACCU GCU CAACUG	1820
2645	UUUGCG AGAA GCAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1709	GUGCA GAU CGCAAA	1821
2677	CCAAGC AGAA GUCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1710	GGACC GUC GCUUGG	1822
2748	CCCAAA AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1711	UCACA GAU UUUGGG	1823
2768	GCACCC AGAA GUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1712	AAACU GCU GGGUGC	1824
2895	CUCCCA AGAA GUCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1713	UGACC GUU UGGGAG	1825
3165	GUUGGA AGAA GUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1714	CUACA GAC UCCAAC	1826
3188	UCAUCC AGAA GGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1715	GCCCU GAU GGAUGA	1827
3225	GUACUC AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1716	AUGCC GAC GAGUAC	1828
3262	UGGAGG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1717	CAGCA GCC CCUCCA	1829
3278	AGGGGA AGAA GUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1718	UCACG GAC UCCCCU	1830

Table IV

3358	UGAUGG AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1719	AAGCU GUC CCAUCA	1831
3376	GCAAGA AGAA GUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1720	AGACA GCU UCUUGC	1832
3394	GGUCUG AGAA GUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1721	AUACA GCU CAGACC	1833
3399	UGUGGG AGAA GAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1722	GCUCA GAC CCCACA	1834
3470	GGAACG AGAA GGUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1723	AACCA GUC CGUUCC	1835
3474	UUUGGG AGAA GACU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1724	AGUCC GUU CCCAAA	1836
3489	AGAGCC AGAA GGCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1725	GGCCC GCU GGCUCU	1837
3510	GUGAUA AGAA GGAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1726	AUCCU GUC UAUCAC	1838
3524	UUCAGA AGAA GAUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1727	AAUCA GCC UCUGAA	1839
3609	GGGCUG AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1728	ACACU GUC CAGCCC	1840
3614	CAGGUG AGAA GGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1729	GUCCA GCC CACCUG	1841
3643	GGGCAG AGAA GUCG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1730	CGACA GCC CUGCCC	1842
3648	CCAGUG AGAA GGGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1731	GCCCU GCC CACUGG	1843
3696	CUGGUA AGAA GGGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1732	ACCCU GAC UACCAG	1844
3759	AUUUUC AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1733	CCACA GCU GAAAAU	1845
3851	GAAAGA AGAA GGAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1734	AUCCA GAC UCUUUC	1846
3931	AAACCA AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1735	CCACA GAC UGGUUU	1847
3955	UGGCUA AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1736	ACACC GAC UAGCCA	1848
4310	CCUUGA AGAA GAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1737	GUUCU GCU UCAAGG	1849
4374	GUACCG AGAA GGCC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1738	GGCCG GAU CGGUAC	1850
4423	GGAAGG AGAA GAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1739	ACUCU GUC CCUUCC	1851
4514	UGGUCC AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1740	CCACU GAU GGACCA	1852
4550	AAACAA AGAA GUCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1741	AGACU GAC UUGUUU	1853
4594	GACAGG AGAA GCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1742	AUGCC GCC CCUGUC	1854
4600	CAGCAA AGAA GGGG	1743	CCCCU GUC UUGCUG	1855

Table IV

	ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA			
4653	GCUGGA AGAA GAGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1744	ACUCG GAU UCCAGC	1856
4660	AAUGUG AGAA GGAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1745	UUCCA GCC CACAUU	1857
4701	AUUCUC AGAA GUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1746	CCACA GCU GAGAAU	1858
4733	AACAAA AGAA GUGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1747	ACACC GCU UUUGUU	1859
4775	CAUUUC AGAA GAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1748	GCUCA GAU GAAAUG	1860
4831	UUUCAG AGAA GCUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1749	AAGCU GCU CUGAAA	1861
4962	GGGGGC AGAA GACC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1750	GGUCA GCU GCCCCC	1862
4965	UUUGGG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1751	CAGCU GCC CCCAAA	1863
5011	ACUCAA AGAA GAGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1752	UCUCU GCC UUGAGU	1864
5040	GGCCAG AGAA GUAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1753	UUACA GCU CUGGCC	1865
5161	UAAAAC AGAA GCAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1754	UUGCA GAU GUUUUA	1866
5277	UAACCA AGAA GGUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1755	AACCU GAC UGGUUA	1867
5292	ACAAAG AGAA GCUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1756	CAGCA GUC CUUUGU	1868
5381	ACUGUA AGAA GAAA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1757	UUUCA GCC UACAGU	1869
5453	UGACUG AGAA GGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA	1758	UCCCA GAU CAGUCA	1870

Claims

1. An enzymatic nucleic acid molecule which specifically cleaves RNA derived from an epidermal growth factor receptor (EGFR) gene.

2. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid molecule is in a hairpin motif.

3. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid molecule is in a hammerhead motif.

4. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises a stem II region of length greater than or equal to 2 base pairs.

5. The enzymatic nucleic acid molecule of claim 3, wherein the binding arms of said nucleic acid molecule comprises sequences complementary to any of SEQ ID NOs 1-823.

6. The enzymatic nucleic acid molecule of claim 2, wherein the binding arms of said nucleic acid molecule comprises sequences complementary to any of SEQ ID NOs 1759-1870.

7. The enzymatic nucleic acid molecule of claim 2, wherein said nucleic hairpin motif consists essentially of any ribozyme sequence shown as SEQ ID NOs 1647-1758.

8. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic hammerhead motif consists essentially of any ribozyme sequence shown as SEQ ID NOs 824-1646.

9. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid molecule is in a hepatitis delta virus, VS nucleic acid, group I intron, Group II intron, or RNase P nucleic acid motif.

10. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid comprises between 12 and 100 bases complementary to said RNA.

11. The enzymatic nucleic acid molecule of claim 1, wherein said nucleic acid comprises between 14 and 24 bases complementary to said mRNA.

12. A mammalian cell including an enzymatic nucleic acid molecule of claim 1.

13. The cell of claim 12, wherein said cell is a human cell.

14. An expression vector comprising nucleic acid sequence encoding at least one of the enzymatic nucleic acid molecule of claim 1, in a manner which allows expression of that enzymatic nucleic acid molecule.

15. A mammalian cell including an expression vector of claim 14.

16. The cell of claim 15, wherein said cell is a human cell.

17. A method for treatment of cancer comprising the step of administering to a patient the enzymatic nucleic acid molecule of claim 1.

18. A method for treatment of a cancer comprising the step of administering to a patient the expression vector of claim 14.

19. A method for treatment of cancer comprising the steps of: a) isolating cells from a patient; b) administering to said cells the enzymatic nucleic acid molecule of claim 1 or 14 ; and c) introducing said cells back into said patient.

20. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of claim 1.

21. A method of treatment of a patient having a condition associated with the level of EGFR, wherein said patient is administered the enzymatic nucleic acid molecule of claim 1.

22. A method of treatment of a patient having a condition associated with the level of EGFR, comprising contacting cells of said patient with the nucleic acid molecule of claim 1, and further comprising the use of one or more drug therapies.

23. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of the 5' terminal nucleotides, and wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3' end modification.

24. The enzymatic nucleic acid of claim 22, wherein said nucleic acid comprises a 3'-3' linked inverted ribose moeity at said 3' end.

25. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises at least five ribose residues, and wherein said nucleic acid molecule comprises phosphorothioate linkages at at least three of the 5' terminal nucleotides, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid molecule comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3' end modification.

26. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises at least five ribose residues, and wherein said nucleic acid molecule comprises phosphorothioate linkages at at least three of the 5' terminal nucleotides, and wherein said nucleic acid molecule comprises an abasic substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid molecule comprises a 3'-end modification.

27. The enzymatic nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises of at least five ribose residues, and wherein said nucleic acid comprises phosphorothioate linkages at at least three of the 5' terminal nucleotides, and wherein said nucleic acid molecule comprises a 6-methyl uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid molecule

comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid molecule comprises a 3' end modification.

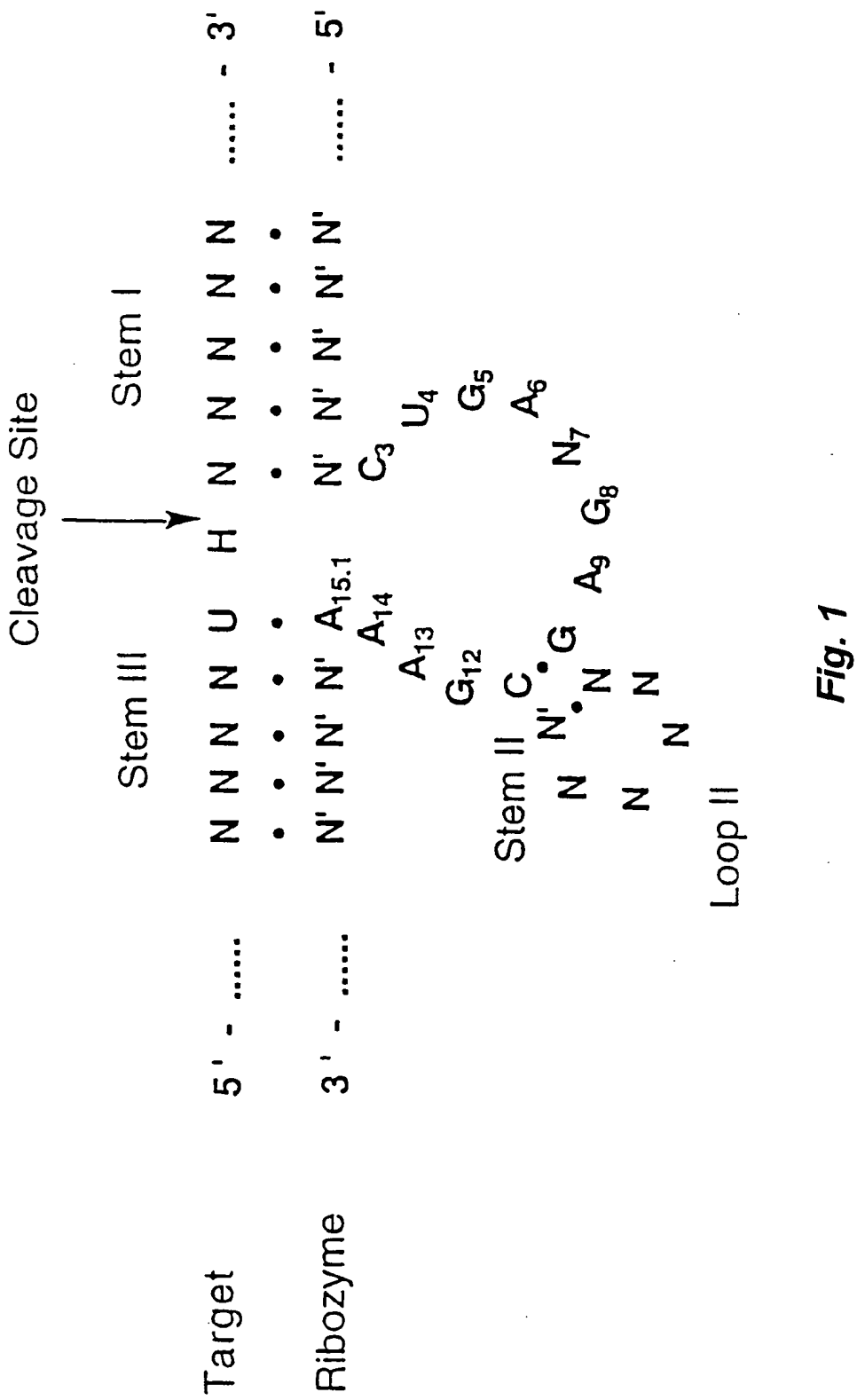

Cleavage Site
Stem I
Stem III
Stem II
Loop II
Target
Ribozyme
5' - .....
3' - .....
..... - 3'
..... - 5'
N N N N U H N N N N N
N' N' N' N' A15.1 N' N' N' N'
A14
A13
G12
C3
U4
G5
A6
N7
G8
A9
C
N'
G
N
N
N
N
N

Fig. 1

Fig. 2A

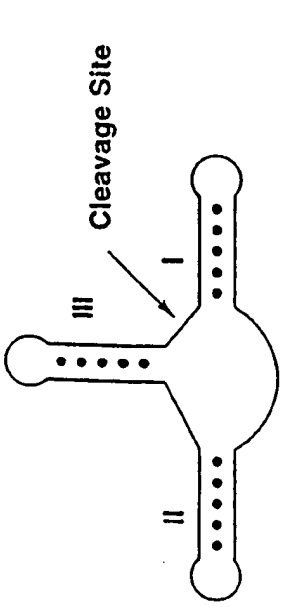


Fig. 2B

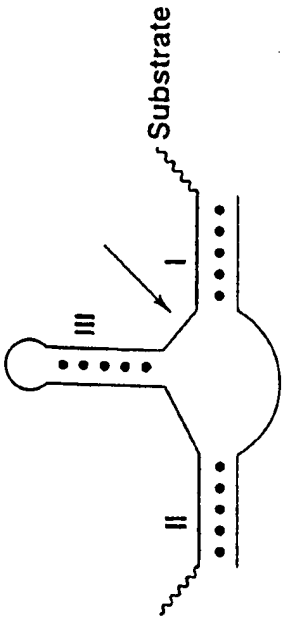


Fig. 2C

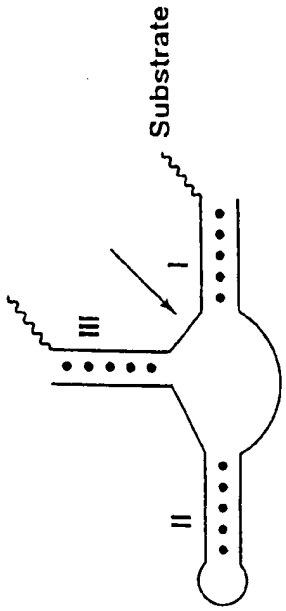
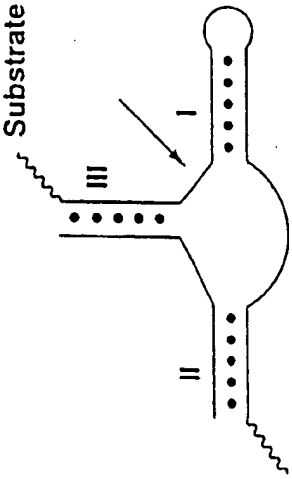


Fig. 2D



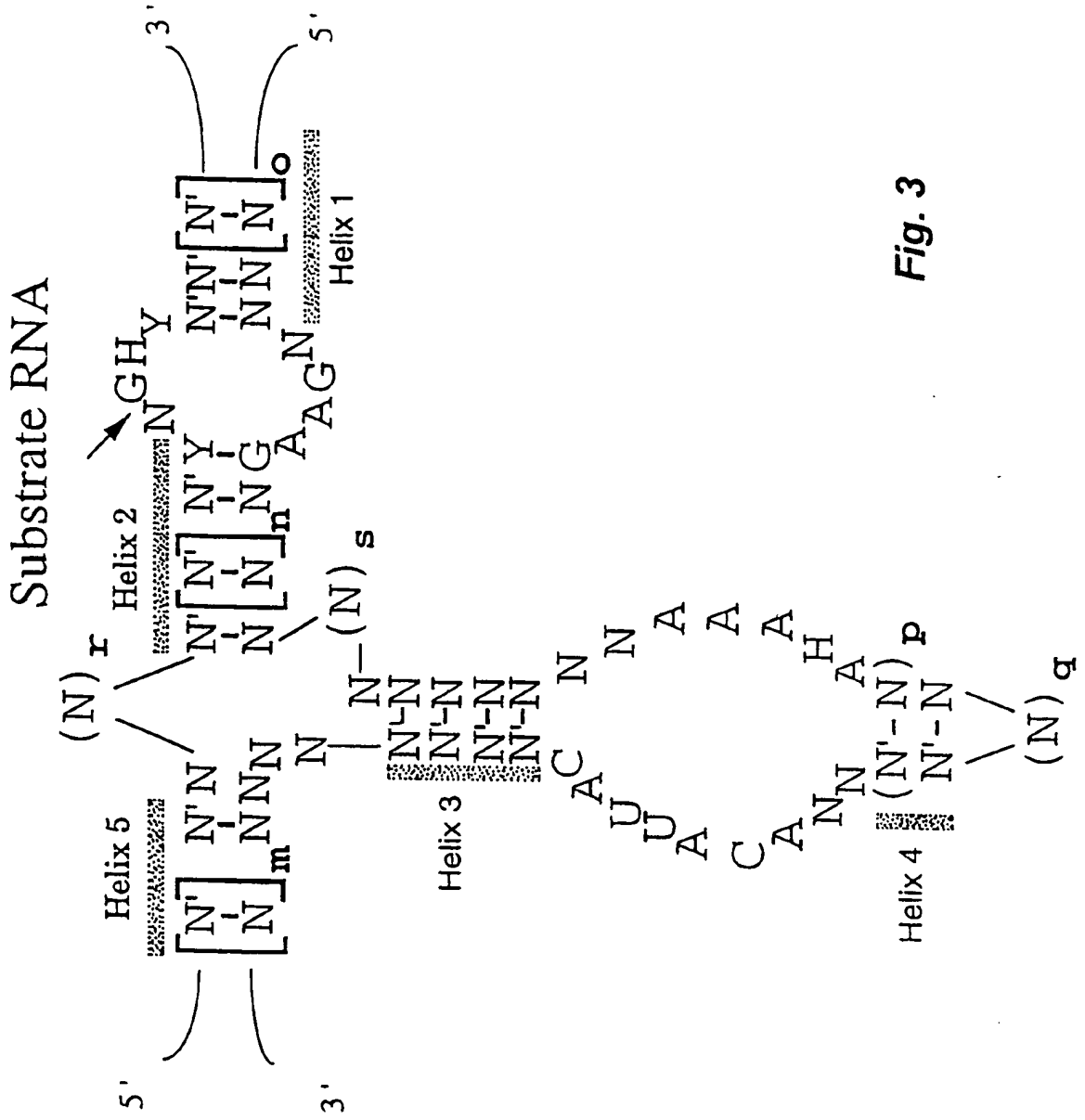


Fig. 3

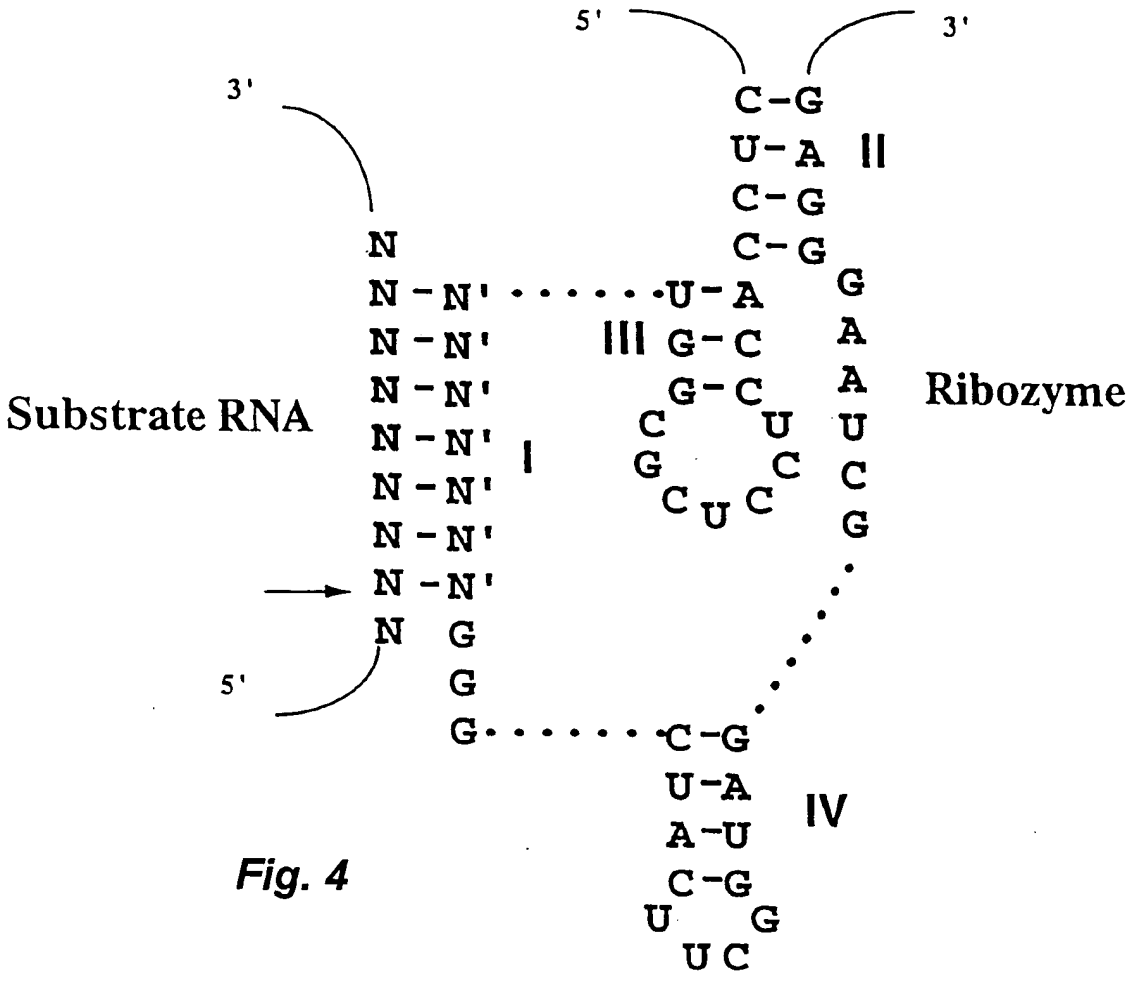


Fig. 4

Fig. 5

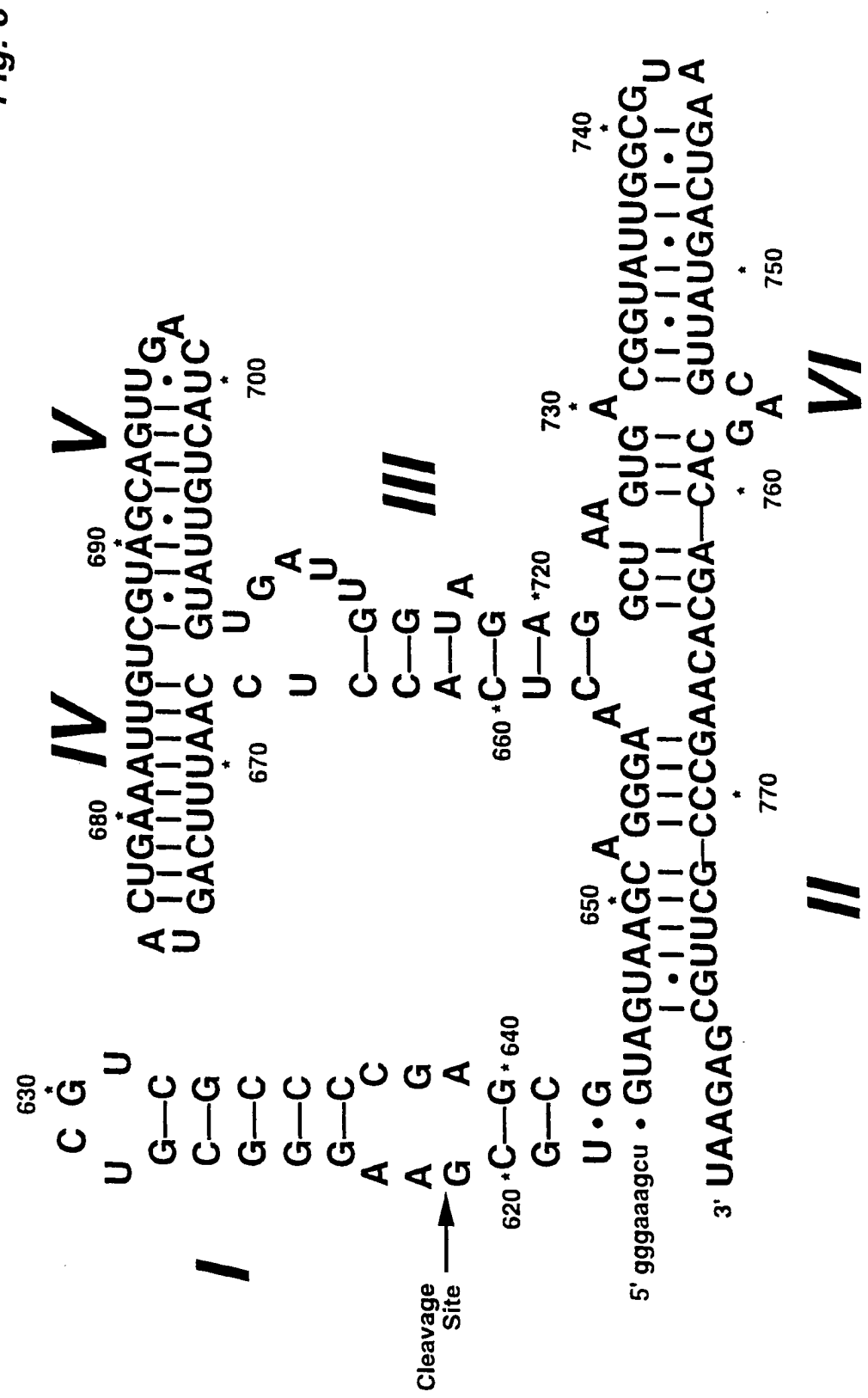
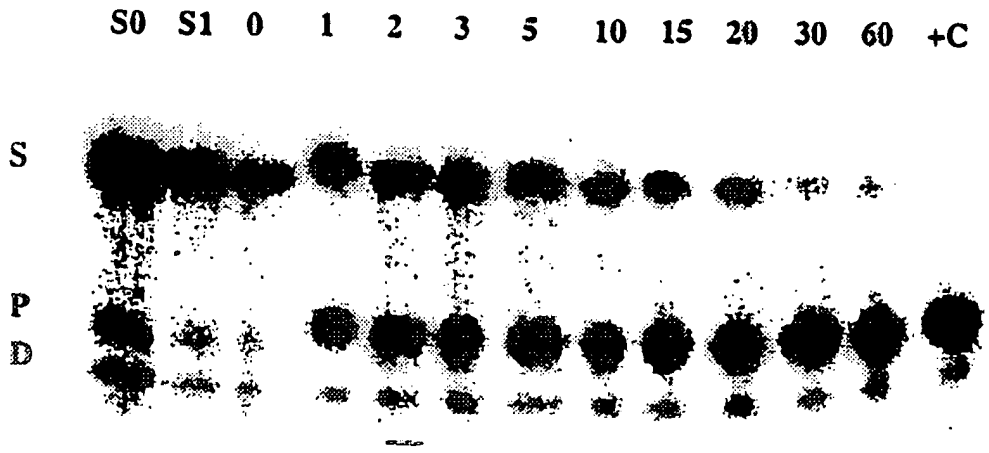


FIG. 6A.



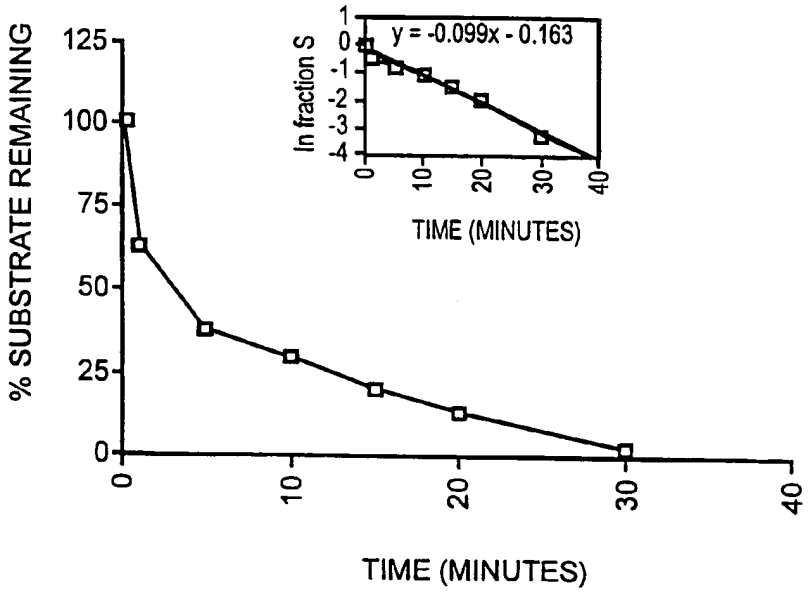
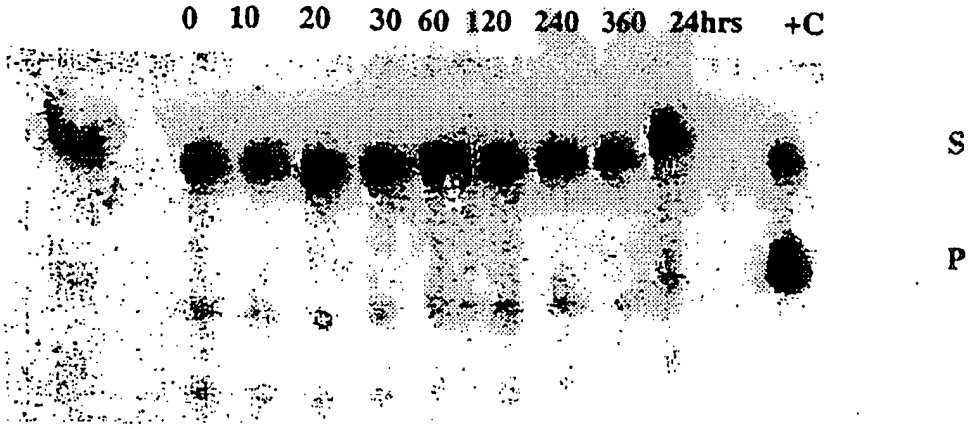

% SUBSTRATE REMAINING
125
100
75
50
25
0
0
10
20
30
40
TIME (MINUTES)
ln fraction S
y = -0.099x - 0.163
1
0
-1
-2
-3
-4
0
10
20
30
40
TIME (MINUTES)

Fig. 6B

FIG. 6C.



10nM ribozyme : 300nM substrate

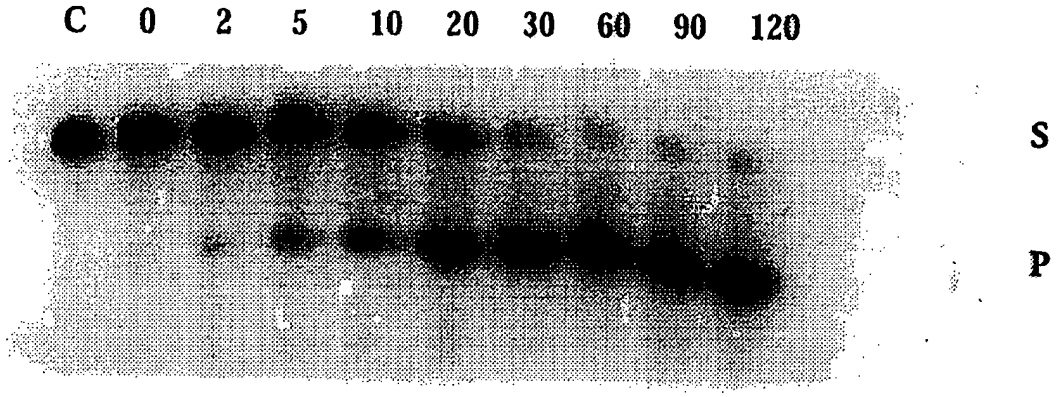


FIG. 7A.

10nM ribozyme : 1μM substrate

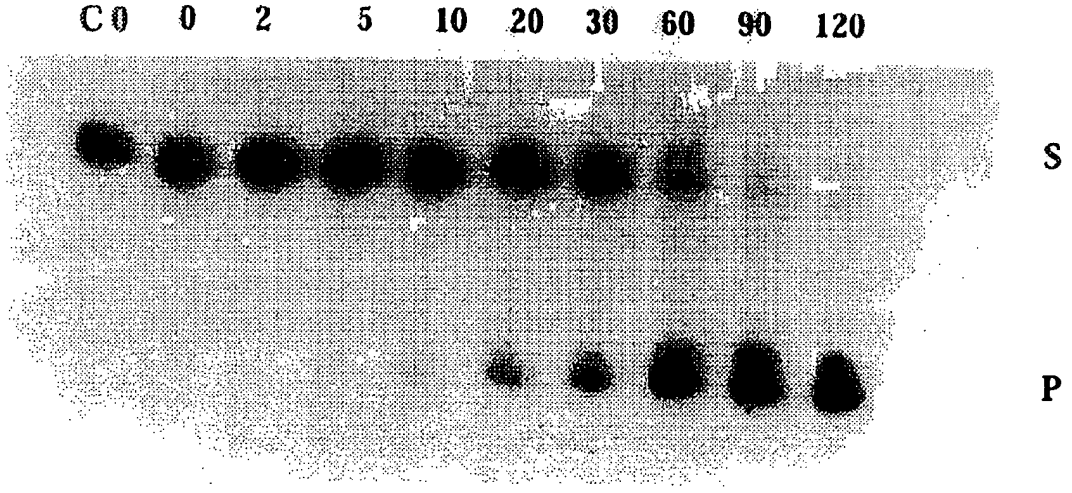


FIG. 7B.

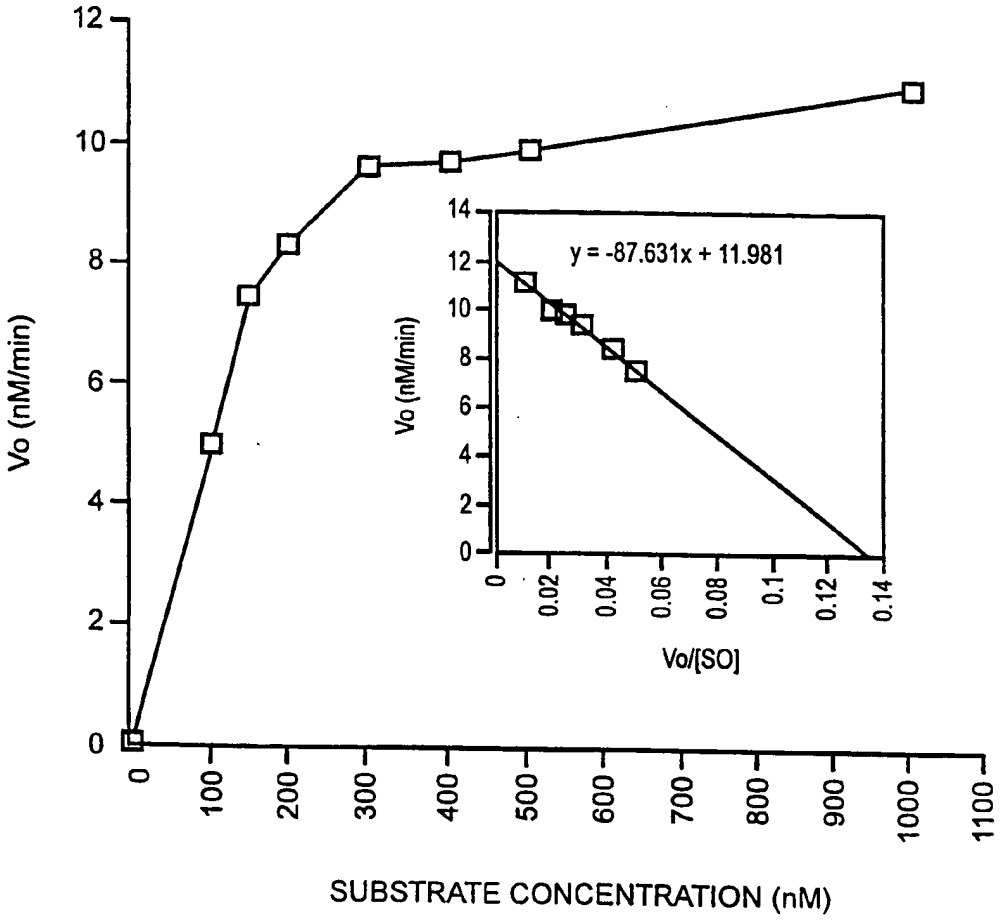

12
10
8
6
4
2
0
Vo (nM/min)
0
100
200
300
400
500
600
700
800
900
1000
1100
SUBSTRATE CONCENTRATION (nM)
y = -87.631x + 11.981
14
12
10
8
6
4
2
0
Vo (nM/min)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
Vo/[SO]

Fig. 7C

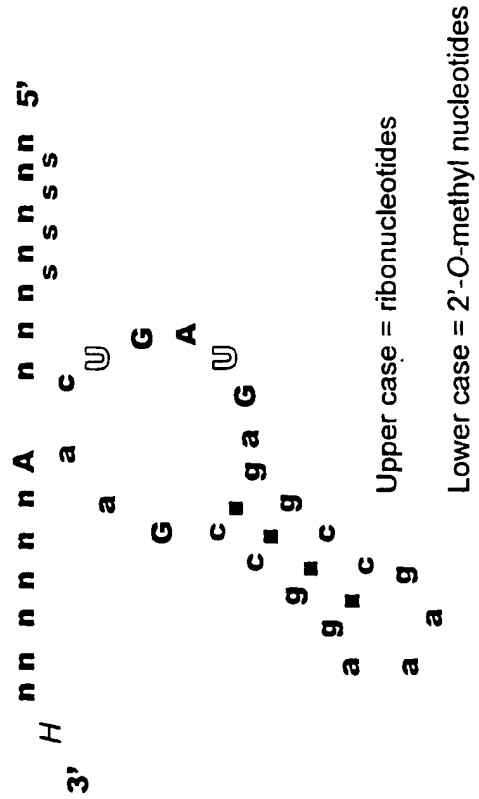


Fig. 8

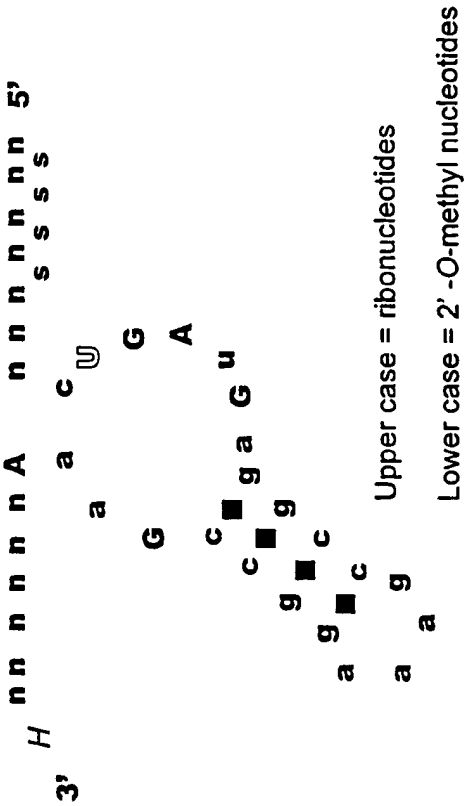


Fig. 9